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UTILITY PATENT APPLICATION TRANSMITTAL (Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
500.1004CONTotal Pages in this Submission
197**TO THE ASSISTANT COMMISSIONER FOR PATENTS**Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

PREPARATION FOR THE APPLICATION OF AGENTS IN MINI-DROPLETS

and invented by:

Gregor CEVC

If a **CONTINUATION APPLICATION**, check appropriate box and supply the requisite information:☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: 07/844,664

Which is a:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.:

Which is a:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.:

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 159 pages and including the following:
 - a. ☒ Descriptive Title of the Invention
 - b. ☐ Cross References to Related Applications (if applicable)
 - c. ☐ Statement Regarding Federally-sponsored Research/Development (if applicable)
 - d. ☐ Reference to Microfiche Appendix (if applicable)
 - e. ☒ Background of the Invention
 - f. ☒ Brief Summary of the Invention
 - g. ☒ Brief Description of the Drawings (if drawings filed)
 - h. ☒ Detailed Description
 - i. ☒ Claim(s) as Classified Below
 - j. ☒ Abstract of the Disclosure

UTILITY PATENT APPLICATION TRANSMITTAL (Small Entity)

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Application Elements (Continued)

3. ☒ Drawing(s) (when necessary as prescribed by 35 USC 113)

a. ☒ Formal b. ☐ Informal Number of Sheets 21

4. ☒ Oath or Declaration

a. ☐ Newly executed (original or copy) ☐ Unexecuted

b. ☒ Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional application only)

c. ☒ With Power of Attorney ☐ Without Power of Attorney

d. ☐ DELETION OF INVENTOR(S)

Signed statement attached deleting inventor(s) named in the prior application,
see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☒ Incorporation By Reference (usable if Box 4b is checked)

The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche

7. ☐ Genetic Sequence Submission (if applicable, all must be included)

a. ☐ Paper Copy

b. ☐ Computer Readable Copy

c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☒ Assignment Papers (cover sheet & documents)

9. ☐ 37 CFR 3.73(b) Statement (when there is an assignee)

10. ☐ English Translation Document (if applicable)

11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☒ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing

☐ First Class ☒ Express Mail (Specify Label No.): EL 563 210 145 US

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197

Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)
16. ☒ Small Entity Statement(s) - Specify Number of Statements Submitted: 1
17. ☐ Additional Enclosures (please identify below):

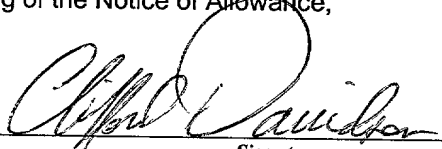
Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	38	- 20 =	18	x \$9.00	\$162.00
Indep. Claims	2	- 3 =	0	x \$39.00	\$0.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$345.00
OTHER FEE (specify purpose)					\$0.00
TOTAL FILING FEE					\$507.00

- ☒ A check in the amount of \$507.00 to cover the filing fee is enclosed.
- ☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. 50-0552 as described below. A duplicate copy of this sheet is enclosed.
- ☐ Charge the amount of as filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: July 21, 2000


Signature
Clifford M. Davidson
Reg. No. 32,728



23280

CC:

PATENT TRADEMARK OFFICE

INDEPENDENT INVENTOR(S)

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY
STATUS (37 C.F.R. 1.9(f) AND 1.27(b)) - INDEPENDENT INVENTOR**

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 C.F.R. 1.9(c) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled PREPARATION FOR THE APPLICATION OF AGENTS IN MINI-DROPLETS described in

- a) ☐ the specification filed herewith.
- b) ☒ application international serial no. PCT/EP91/01596, filed 22 AUGUST 1991.
- c) ☐ patent no. _____, issued _____.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 C.F.R. 1.9(c) if that person has made the invention, or to any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or a nonprofit organization under 37 C.F.R. 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- a) ☐ no such person, concern, or organization
- b) ☐ persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. 1.27)

NAME _____
ADDRESS _____
a) ☐ INDIVIDUAL b) ☐ SMALL BUSINESS CONCERN c) ☐ NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
a) ☐ INDIVIDUAL b) ☐ SMALL BUSINESS CONCERN c) ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereof, or any patent to which this verified statement is directed.

DR. GREGOR CEVC

NAME OF INVENTOR

Signature of Inventor

Date

NAME OF INVENTOR

Signature of Inventor

Date

NAME OF INVENTOR

Signature of Inventor

Date

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Re: Application of: CEVC, Gregor
Serial No.: Not Yet Known
Filed: Herewith
For: PREPARATION FOR THE APPLICATION OF AGENTS
IN MINI DROPLETS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

July 21, 2000

Sir:

Please enter the following amendment before the examination of the above referenced application:

IN THE CLAIMS

Please cancel claims 1-30 without prejudice to applicant's rights to pursue the subject matter in a future continuation or divisional application.

Please add new claims 31-69 as follows:

31. (New) A preparation suitable for transporting active agents through permeability barriers, comprising a plurality of transfersomes in a medium, said transfersomes comprising a pharmaceutically acceptable lipid and a pharmaceutically acceptable surfactant which is compatible with said lipid, the ratio of said lipid to said surfactant enabling said transfersomes to undergo sufficient deformation to enable said transfersomes to pass as an entity through a permeability barrier which has pores smaller than the size of said

transfersomes, wherein the total concentration of said lipid in said medium is from about 0.1% to about 30%, by weight and the ratio of lipid to surfactant is from about 5.5:1 to about 1:500.

32. (New) Preparation as claimed in claim 31, wherein said transfersomes are unilamellar.
33. (New) Preparation as claimed in claim 31, wherein said permeability barrier is mammalian skin.
34. (New) Preparation as claimed in claim 31, wherein the concentration of said surfactant is between 20 and 50 mol-% of the concentration of said surfactant causes said lipid to be solubilized, and the edge tension of said transfersomes is about 10 Piconewton or less.
35. (New) Preparation as claimed in claim 31, further comprising an active agent associated with said transfersomes, said active agent being contained in the interior of said transfersome in an outer membrane of said transfersome, or both.
36. (New) Preparation as claimed in claim 31 wherein the total concentration of said lipid in said medium is between 0.1 and 15 weight-%.
37. (New) Preparation as claimed in claim 31, wherein the total concentration of said lipid in said medium is between 5 and 10 weight-%.
38. (New) Preparation as claimed in claim 31, wherein the total concentration of said lipid in said medium for application on plants is 0.000001 through 10 weight-%.
39. (New) Preparation as claimed in claim 31, wherein the total concentration of said lipid in said medium for application on plants is between 0.001 and 1 weight-%.

40. (New) Preparation as claimed in claim 31, wherein the total concentration of said lipid in said medium for application on plants is between 0.01 and 0.1 weight-%.
41. (New) Preparation as claimed in claim 31 wherein the active agent is selected from the group consisting of an adrenocorticostatic, a β -adrenolytic, an androgen, an antiandrogen, an antiparastic, an anabolic steroid, an anaesthetic, an analgesic, an analeptic, an antiallergic, an antiarrhythmic, an antiarterosclerotic, an antiasthmatic, a bronchospasmolytic, an antibiotic, an antidepressant, an antipsychotic, an antidiabetic, an antidote, an antiemetic, an antiepileptic, an antifibrinolytic, an anticonvulsive, an anticholinergic, an enzyme, a coenzyme, an enzyme inhibitor, an antihistaminic, an antihypertonic, an anticoagulant, an antimycotic, an antimyasthenic, an anti-parkinson agent, an antiphlogistic, an antipyretic, an antirheumatic, an antiseptic, a respiratory analeptic, a respiratory stimulant, a broncholytic, a cardiotonic, a chemotherapeutic, a coronary dilator, a cytostatic, a diuretic, a ganglium-blocker, a glucocorticoid, an anti-viral agent, a haemostatic, a hypnotic, an immunologically active substance, a carbohydrate, a contraceptive, an antimigraine agent, a morphine-antagonist, a muscle relaxant, a narcotic, a nucleotide, a neuroleptic, a neurotransmitter, a neurotransmitter antagonist, an ophthalmic agent, a sympathicomimetic, a sympathicoclytic, a parasympaticomimetic, a parasympathicolytic, a protein, a protein derivative, an anti-psoriasis agent, a neurodermitis drug, a mydriatic, a psychostimulant, a rhinologic, a sleep-inducing agent, a stimulant, a sedating agent, a spasmolytic, a tuberlostatic, a urologic, a vasoconstrictor, a vasodilator, a virustatic, a wound-healing substance, and a combination thereof.
42. (New) Preparation as claimed in claim 35 wherein said active agent is a growth modulating substance for living organisms.

43. (New) Preparation as claimed in claim 35 wherein said active agent exerts biocidal activity as an insecticide, pesticide, herbicide or fungicide.
44. (New) Preparation as claimed in claim 35 wherein the active agent is an attractant.
45. (New) Preparation as claimed in claim 35 wherein the active agent is a pheromone.
46. (New) A method of manufacturing preparations for the transport of agents through permeability barriers:
 - (A) combining a lipid and a surface active agent that can solubilize said lipid in a suitable medium and determining the ratio of lipid to surface active agent which enables transfersomes formed by combining said lipid and said surface active agent in said medium to undergo sufficient deformation to enable said transfersomes to pass as an entity through a permeability barrier which has pores smaller than the size of said transfersomes, and
 - (B) preparing said transfersomes in said medium such that the total concentration of said lipid in said medium is from about 0.1% to about 30%, by weight.
47. (New) Method as claimed in claim 46 wherein said transfersomes are unilamellar .
48. (New) Method as claimed in claim 46, further comprising determining the stability and the permeation capacity of said transfersomes the droplets by means of gravity or pressure filtration, through a fine-pore filter.
49. (New) Method of claim 47 wherein the stability and the permeation capacity of said transfersomes are determined by means of mechanical fragmentation.

50. (New) Method as claimed in claim 46 wherein the content of said surface active substance is between 20 and 50 mol-% of the concentration of such substance that causes said lipid to be solubilized.
51. (New) Preparation as claimed in claim 33 wherein said preparation comprises at least one antidiabetic agent.
52. (New) Preparation as claimed in claim 46 wherein said transfersomes have a double layer structure.
53. (New) Preparation as claimed in claim 46, wherein said lipid is a synthetic lipid.
54. (New) Preparation as claimed in claim 46, wherein said lipid comprises a glyceride.
55. (New) Preparation as claimed in claim 46, wherein said lipid is selected from the group consisting of glycerophospholipid, isoprenoidlipid, sphingolipid, a sulfur-containing lipid, and a carbohydrate-containing lipid.
56. (New) Preparation as claimed in claim 46, wherein said lipid comprises a fatty acid.
57. (New) Preparation as claimed in claim 46, wherein said lipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidic acid, phosphatidylserine, sphingomyeline, sphingophospholipid, glycosphingolipid, cerebroside, ceramidepolyhexoside, sulfatide, sphingoplasmalogene, a ganglioside, and a glycolipid.
58. (New) Preparation as claimed in claim 46, wherein said lipid is selected from the group consisting of dioleoyl lipid, dilinoleyl lipid, dilinolenyl lipid, dilinolenoyl lipid, diarachidoyl lipid, dimyristoyl lipid, dipalmitoyl lipid, distearoyl lipid, phospholipid,

diacyl lipid and dialkyl lipid.

59. (New) Preparation as claimed in claim 31, wherein surfactant is selected from the group consisting of nonionic surfactants, zwitterionic surfactants, anionic surfactants and cationic surfactants.
60. (New) Preparation as claimed in claim 31, wherein said surfactant is selected from the group consisting of a long-chain fatty acid, a long-chain fatty alcohol, an alkyl-trimethyl-ammonium-salt, an alkylsulfate salt, a cholate-, a deoxycholate-, a glycodeoxycholate-, taurodeoxycholate, dodecyl-dimethyl-aminoxide, decanoyl-N-methylglucamide, dodecanoyl-N-methylglucamide, N-dodecyl-N, N-dimethylglycine, 3-(hexadecyldimethylammonio)-propane-sulfonate, N-hexadecyl-sulfobetaine, nonaethylene-glycol-octylphenylether, nonaethylene-dodecylether, octaethyleneglycol-isotridecylether, octaethylenedodecylether, polyethylene glycol-20-sorbitanemonolaurate, polyhydroxyethylene-cetylstearyl ether polyhydroxyethylene-4-laurylether, polyhydroxyethylene-23-laurylether, polyhydroxyethylene-8-stearate, polyhydroxyethylene-40-stearate, polyhydroxyethylene-100-stearate, polyethoxylated castor oil 40, polyethoxylated hydrated castor oil, sorbitanemonolaurate, lauryl-salts, oleoylsulfate-salts, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium elaidate, sodium linoleate, sodium laurage, nonaethylene-dodecylether, polyethylene glycol-20-sorbitane-monooleate, polyhydroxyethylene-23-laurylether, polyhydroxyethylene-40-stearate, a sorbitane phospholipid, a monolaurate phospholipid, and a lysophospholipid.
61. (New) Preparation as claimed in claim 35, wherein said agent comprises 1 through 500 I.U. insulin/ml.

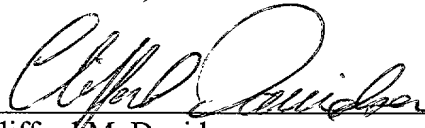
62. (New) Preparation as claimed in claim 35, wherein said agent comprises between 20 and 100 I.U. insulin/ml .
63. (New) Preparation as claimed in claim 31, wherein the total concentration of said lipid in the preparation is between 0.1 through 20 weight-%.
64. (New) Preparation as claimed in claim 31, wherein the total concentration of said lipid in the preparation is between 0.5 and 15 weight-%.
65. (New) Preparation as claimed in claim 31, wherein the concentration of said lipid in the preparation is between 2.5 and 10 weight-%.
66. (New) Preparation as claimed in claim 31, wherein said lipid is selected from the group consisting of phosphatidylcholine and phosphatidylglycol.
67. (New) Preparation as claimed in claim 31, wherein said surfactant is selected from the group consisting of lysophosphatidic acid, lysophosphoglycerol, deoxycholate, glycodeoxycholate, laurate, myristate, oleate, palmitoleate, phosphate salts thereof, sulfate salts thereof, a Tween-surfactant and a Myrj-surfactant.
68. (New) Preparation as claimed in claim 31, wherein the radius of said transfersomes in the preparation is between approximately 50 and approximately 200 nm.
69. (New) Preparation as claimed in claim 31 wherein the radius of said transfersomes in the preparation is between approximately 100 and approximately 180 nm.

REMARKS

Applicant respectfully request entry of this amendment and an early and favorable action on the merits is earnestly solicited.

The Examiner is requested to contact the undersigned if a telephone interview will advance the prosecution of this case.

Respectfully submitted,
DAVIDSON, DAVIDSON & KAPPEL, LLC



Clifford M. Davidson
Reg. No. 32,728

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Preparation for the application of agents in mini-droplets

The present invention relates to a novel type of preparations suitable for the application of different agents in the form of a minuscule droplet or, in particular, a vesicle consisting of one or a few membrane-like amphiphile assemblies. These can mediate the transport of agents into and through a series of natural permeability barriers or through the constrictions in such barriers; for example, through intact skin or similar organs. The invention further relates to a procedure for the large-scale production of such carriers. As a special example, non-invasive application of antidiabetics is described for the case of insulin.

The application of various agents is often hampered by the presence of barriers with a low permeability to such agents. Owing to skin impermeability, for example, many common therapeutic agents must be applied per os or parenterally (i.v., i.m., i.p.). Intrapulmonary and intranasal applications of aerosols, the use of rectal formulations, gels for mucous applications, or use of ocular formulations are only practicable in certain areas and not for all types of drugs. The transport of different agents into plant tissues is subject to even more severe constraints due to the high permeability barrier of the cuticular wax layers.

Noninvasive drug application through permeability barriers thus would be advantageous in many cases. In humans and animals one would expect such a percutaneous application of agents to protect the agents from degradation in the gastrointestinal tract; modified drug distribution could possibly also be achieved. Such drug application, moreover, would influence the pharmacokinetics of the agent molecules and permit simple as well as multiple noninvasive therapy.

(Karzel K., Liedtke, R.K. (1989) *Arzneim. Forsch./Drug Res.* 39, 1487-1491). In the case of plants, improved penetration

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into or through the cuticle could reduce the drug concentration required for a given application and thus significantly diminish pollution problems (Price, C.E. (1981) In: The plant cuticle (D.F. Cutler, K.L. Alvin, C.E. Price, Edits.), Academic, New York, pp. 237-252).

There are many reports on different attempts to increase the permeability of intact skin by suitable manipulations (cf. Karzel und Liedtke, op. cit.). Jet injection (Siddiqui & Chien (1987) Crit. Rev. Ther. Drug. Carrier. Syst. 3, 195-208.), the use of electric fields (Burnette & Ongpipattanakul (1987) J. Pharm. Sci. 76, 765-773) or chemical penetration enhancers, such as solvents and surfactants, are particularly worth mentioning. A long list of additives which have been used to enhance the penetration of one particular water soluble agent (Nolaxon) into skin, for example, is given in the work by Aungst et al. (1986, Int. J. Pharm. 33, 225-234). This list encompasses nonionic substances (including long-chain alcohols, surfactants, zwitterionic phospholipids, etc.), anionics (most notably fatty acids), cationic long-chain amines, sulfoxides as well as different amino-derivatives; amphoteric glycinates and betaines are also mentioned. Despite all this, the problem of agent penetration into skin has as yet not at all - or not satisfactorily - been solved.

A survey of procedures used for increasing the penetration of agents through a plant cuticle is given in the work by Price (1981, op.cit.). To date it has been common to simply add chemical penetration enhancers to the mixture of agent and other molecules; applications to human skin were the only case in which additives were sometimes applied in advance, in the form of an organic solution. The reason for this application form was the current concept for the action of penetration enhancers: to date one has studied, discussed, and believed

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that, in general, any facilitated agent penetration is a consequence of skin fluidization, on the one hand (Golden et al., (1987) J. Pharm. Sci. 76, 25-28). (This phenomenon is normally associated with a destruction of the skin surface and of its protective shield and thus is undesired.) On the other hand, it has been shown that some agents can permeate through skin in the form of low-molecular weight complexes with added molecules (Green et al., (1988) Int. J. Pharm. 48, 103-111).

Methods deviating from the ones already described have brought little improvement to date. The use of lipoidal carriers, the liposomes, on intact skin, which has been theoretically discussed by several authors, was mainly aimed at modifying the agent's pharmacokinetics (Patel, Bioch. Soc. Trans., 609th Meeting, 13, 513-517, 1985, Mezei, M. Top. Pharm. Sci. (Proc. 45th Int. Congr. Pharm. Sci.F.I.P.) 345-58 Elsevier, Amsterdam, 1985). Thus far, all proposal of this kind, moreover, involved the use of standard lipid vesicles (liposomes) which cannot penetrate the skin at all or permeate through the skin very inefficiently, as is shown in this patent application. Patent applications nos. JP 61/271204 A2 [86/271204] refer to a related use of liposomes in which hydrochinonglucosidal is employed to improve the stability of the agent.

Hitherto available preparations for percutaneous use have mostly been applied under occlusion; in the case of liposomal preparations, this was even a general rule. The corresponding preparations only contained small or lipophilic substances, as well as a limited number of skin-fluidizing additives. Correspondingly, they afforded only partial control over the pharmacokinetic properties of final preparations. In an attempt to improve this situation a proposal was made (WO 87/1938 A1) to use drug-carrying lipid vesicles in combination with a gelatinizing agent as a transdermal patch. This has

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By a specially designed formulation we have succeeded in obtaining results which were dramatically better than those of Gesztes and Mezei. Our carrier formulations consisted of filtered lipid vesicles (liposomes) which also contained some detergents, with a declared optimum lipid/surfactant content of 1-40/1, in practice mainly around 4/1.

Since then, we have unexpectedly discovered that certain criteria, described in this application, may be formulated for the qualification of drug carriers as suitable for the penetration into and through a permeability barrier. The main requirement of such a drug carrier - which in the following is called a transfersome - is that it is sufficiently elastic to penetrate through the constrictions in a barrier, such as skin. In the case of transfersomes consisting of phosphatidylcholine and sodium cholate this condition is fulfilled when the edge tension of a carrier is below 10 Piconewton; similar values are also likely to pertain to other, related systems. Carriers which are capable of creating a gradient after an application are particularly

It is, therefore, an object of the present invention to specify the properties of novel preparations which are suitable for the mediation of rapid transport of diverse agents and other substances through permeability barriers and constrictions.

It is yet another object of this invention to prepare formulations for non-invasive application of antidiabetics, most notably of insulin; these should ensure an improved, therapeutically sufficient, and reproducible form of drug application.

These objects have been accomplished through the features of the independent claims.

The transfersomes according to this invention differ from the liposomes hitherto described for topical application and from other related carriers in at least three basic features.

Firstly, they can consist of an arbitrary amphiphile, including oils. Secondly, they can be made in arbitrary fashion: their penetration capacity does not depend on the

Transfersomes also differ from micellar carrier formulations in at least two basic features. Firstly, a transfersome is, as a rule, far bigger than a micelle; consequently, it also obeys different diffusion laws. Secondly, and more importantly, a transfersome typically contains a water-filled central core (the inner lumen of a vesicle). Nearly all water soluble substances can be incorporated in the core of a transfersome and thus transported across a permeability barrier. Transfersomes are suitable for transporting amphiphilic and lipophilic substances.

If simple carriers are not sufficiently deformable and their permeation capacity must be achieved by using certain edge-active additives, the concentration of the latter is then preferably in the range between 0.1 and 99 % of the quantity which would be required for carrier solubilization. Frequently, the optimum - depending on the purpose and the drug used - is located in the range between 1 and 80 %, most frequently between 10 and 60 % of the solubilization dose; the

concentration range between 20 and 50 mol-% is the most preferred dose.

Our novel transfersomes can mediate transport of agents through essentially all permeability barriers and are suitable, for example, for percutaneous (dermal) applications of medical agents. Transfersomes can carry water- or fat-soluble agents to various depths at the application site, depending on the transfersomal composition, application dose, and form. Special properties which cause a carrier to behave as a transfersome can be realized for phospholipid vesicles as well as for other types of amphiphile aggregates.

In this application it is shown for the first time that by means of suitably formulated transfersomes, a major proportion of the drugs applied can be introduced not only into a permeability barrier, such as skin, but, moreover, can be transported into the deeper tissues where they become systemically active. Transfersomes can carry polypeptides, for example, through intact skin at an effectiveness which is a 1,000 times higher than was previously possible when using structureless penetration enhancers. Transfersomally formulated substances can reach nearly 100 % of the corresponding biological or therapeutical maximum efficacy after applications on human skin. Similar effects, to date, have only been achievable by using an injection needle.

In the course of this study, it has surprisingly been found that through use of such novel drug carriers, antidiabetics can be brought into the blood through intact skin without the necessity of auxiliary measures such as an injection. After a dermal application of insulin applied in the form of transfersomes, more than 50 % and often more than 90 % of the applied drug dose are routinely found in the destined organs of the body. Insulin-containing, dermally applied

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The present invention, consequently, opens up a way for simple, noninvasive and completely painless therapy of type II diabetes: transfersomes can be used alone or in combination with an arbitrary dosing means for non-problematic therapy of acute and/or chronic diabetes.

Transfersomes thus offer an elegant, uniform and generally useful means of transport across permeability barriers for diverse agents. These newly developed carriers are perfectly suited for use in human and animal medicine, dermatology, cosmetics, biology, biotechnology, agrotechnology and other fields.

A transfersome according to this invention comprises any carrier with a special capability to get or diffuse into or through a permeability barrier under the effect of a gradient and by so doing to transport material between the application and destination sites.

For dermal applications, for example, preferably particles or vesicles with a diameter of the order of 100-10,000 nm, frequently in the range of 100 to 400 nm, and most frequently with sizes between 100 and 200 nm are used as carriers.

DEFINITIONS

A lipid in the sense of this invention is any substance with characteristics similar to those of fats or fatty materials. As a rule, molecules of this type possess an extended apolar

region (chain, X) and, in the majority of cases, also a water-soluble, polar, hydrophilic group, the so-called head-group (Y). The basic structural formula 1 for such substances reads



where n is greater or equal zero. Lipids with n=0 are called apolar lipids; those with n ≥ 1 are polar lipids. In this context, all amphiphiles, such as glycerides, glycerophospholipids, glycerophosphinolipids, glycerophosphonolipids, sulfolipids, sphingolipids, isoprenoidlipids, steroids, sterines or sterols and lipids containing carbohydrate residues, can simply be referred to as lipids.

A phospholipid, for example, is any compound of formula 2



In this formula, n and R₄ have the same significance as in formula 8 except that R₁ and R₂ cannot be hydrogen, an OH-group or a short chain alkyl residue; R₃ is a hydrogen atom or an OH-group, in the majority of cases. In addition, R₄ can be a short chain alkyl group substituted by three short chain alkylammonium residues, e.g. trimethylammonium, or an amino-substituted short chain alkyl, e.g. 2-trimethylammonioethyl (cholinyl).

A lipid is preferably any substance according to formula 2, in which n=1, R₁ and R₂ is hydroxyacyl, R₃ is a hydrogen atom and R₄ is a 2-trimethylammonioethyl (the last compound corresponding to the phosphatidylcholine headgroup), 2-dimethylammonioethyl, 2-methylammonioethyl or 2-aminoethyl (corresponding to

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A lipid of this kind is, for example, phosphatidylcholine from natural sources, in the old nomenclature also called lecithin. This can be obtained, for example, from eggs (then being rich in arachidic acid), soy-bean (rich in C-18 chains), coconuts (rich in saturated chains), olives (rich in monounsaturated chains), saffron, safflower and sunflowers (rich in n-6 linolenic acid), linseed (rich in n-3 linolenic acid), from whale-oil (rich in monounsaturated n-3 chains), from *Nachtkerze* or primrose (rich in n-3 chains), etc. Preferred natural phosphatidylethanolamines (in the old nomenclature also called cephalins), frequently stem from egg or soy-beans.

Further preferred lipids are synthetic phosphatidylcholines (R_4 in formula 2 corresponding to 2-trimethylammonioethyl), synthetic phosphatidylethanolamines (R_4 being identical to 2-aminoethyl), synthetic phosphatidic acids (R_4 being a proton) or their esters (R_4 corresponding e.g. to a short chain alkyl, such as methyl or ethyl), synthetic phosphatidylserines (R_4 corresponding to an L- or D-serine), or synthetic phosphatidyl(poly)alcohols, such as phosphatidylglycerol (R_4 being identical to L- or D-glycerol). In this case, R_1 and R_2 are identical acyloxy residues such as lauroyl, oleoyl, linoyl, linoleoyl or arachinoyl, e.g. dilauroyl-, dimyristoyl-, dipalmitoyl-, distearoyl-, diarachinoyl-, dioleoyl-, dilinoyl-, dilinoleoyl-, or diarachinoylphosphatidylcholine or -ethanolamine, or different acyl residues, e.g. R_1 = palmitoyl and R_4 = oleoyl, e.g. 1-palmitoyl-2-oleoyl-3-glycerophosphocholine; or different hydroxyacyl residues, e.g. R_1 = hydroxypalmitoyl and R_4 = hydroxyoleoyl; or mixtures thereof, e.g. R_1 = hydroxypalmitoyl and R_4 = oleoyl etc. R_1 can also signify an alkenyl and R_2 identical hydroxyalkyl residues, such as tetradecylhydroxy or hexadecylhydroxy, e.g.

in ditetradecyl- or dihexadecylphosphatidylcholine or -ethanolamine, R_1 can be an alkenyl and R_2 a hydroxyacyl, e.g. a plasmalogen (R_4 = trimethylammonioethyl), or R_1 can be an acyl, e.g. myristoyl, or palmitoyl, and R_2 a hydroxy, e.g. in natural or synthetic lysophosphatidylcholines or lysophosphatidylglycerols or lysophosphatidylethanolamines, e.g. 1-myristoyl- or 1-palmitoyllysophosphatidylcholine or -phosphatidylethanolamine; R_3 is frequently hydrogen.

A convenient lipid according to this invention is also a lipid of the basic formula 2, in which $n=1$, R_1 is an alkenyl residue, R_2 is an acylamido residue, R_3 is a hydrogen atom and R_4 is 2-trimethylammonioethyl (choline residue). A lipid of this kind is known under the term sphingomyeline.

Furthermore, suitable lipids are analogs of lysophosphatidylcholine, such as 1-lauroyl-1,3-propandiol-3-phosphorylcholine, monoglycerides, such as monoolein or monomyristin, a cerebroside, a ganglioside or a glyceride which contain no free or esterified phosphoryl- or phosphono group or a phosphino group in the position 3. One example of such glyceride is diacylglyceride or 1-alkenyl-1-hydroxy-2-acylglyceride with arbitrary acyl or alkenyl groups, the 3-hydroxy group in these then being ether-bonded to one of the mentioned carbohydrate residues, such as a galactosyl residue, for example in monogalactosylglycerol.

Lipids with desired head or chain group properties can also be prepared biochemically, using e.g. phospholipases (such as phospholipase A1, A2, B, C, and especially D), desaturases, elongases, acyl-transferases, etc., starting with any natural or synthetic precursor.

Suitable lipids, furthermore, are all lipids found in

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biological membranes and extractable with suitable apolar organic solvents, such as chloroform. In addition to the lipids already mentioned, this group of lipids also encompasses steroids, such as oestradiols, or sterines, such as cholesterin, beta-sitosterine, desmosterine, 7-keto-cholesterin or beta-cholestanol, fat-soluble vitamins, such as retinoids, vitamins, such as vitamin A1 or A2, vitamin E, vitamin K, such as vitamin K1 or K2, or vitamin D1 or D3, etc.

EDGE ACTIVE SUBSTANCES

An edge active substance according to this application is any substance which is capable of inducing or increasing the carrier system's capacity to form edges, protrusions or relatively strongly curved surfaces; this property also manifests itself in the capability to induce pores in lipid structures, such as membranes, or even provoke a solubilization (lysis) in the higher concentrations ranges. More strictly speaking, all such substances are considered edge-active which exhibit a tendency to accumulate at or near the edges between the polar and apolar parts of molecules and/or near or at the edges between the polar and apolar parts of the supramolecular aggregates, thereby lowering the free energy for the formation of edges and/or strongly curved surfaces. All surfactants and many solvents as well as asymmetric, and thus amphiphatic, molecules or polymers, such as many oligo- and polycarbohydrates, oligo- and polypeptides, oligo- and polynucleotides or their derivatives also belong to this category.

The edge activity of the used 'solvents', surfactants, lipids, or agents depends on the effective relative hydrophilicity or hydrophobicity of each molecule, and can also be modified by the choice of further system components and boundary conditions in the system (temperature, salt content, pH value,

etc.). Functional groups, such as double bonds in the hydrophobic part of molecules, which lower the hydrophobicity of this molecular region, increase edge activity; elongation or space-demanding substituents in the hydrophobic molecular parts, e.g. in the aromatic part, lower the edge activity of a substance. Charged or strongly polar groups in the headgroup normally increase the edge activity provided that the hydrophobic molecular part has remained the same. Direct connections between the lipophilic and/or amphiphilic system components have the reverse effect.

Solvents which are to some extent edge active only in certain concentration ranges encompass simple, especially short chain, alcohols, such as methanol, ethanol, n-propanol, 2-propen-1-ol (allyl alcohol), n-butanol, 2-buten-1-ol, n-pentanol (amyl alcohol), n-hexanol, n-heptanol, n-octanol and n-decanol; furthermore, iso-propanol, iso-butanol or iso-pentanol. Higher alcohols are even more potent, for example, ethandiol (ethylene glycol), 1,2-propane diol (propylene glycol), 1,3-propane diol, 1,3-butane diol, 2,3-butane diol, propane triol (glycerol), 2-butene-1,4-diol, 1,2,4-butane triol, 1,3,4-butane triol, 1,2,3-butane triol, butane tetraol (erythritol), 2,2-bis(hydroxymethyl)1,3-propane diol (pentaerythritol), 2,4-pentadiol and other pentadiols or pentendiols, 1,2,5-pentantriol and other pentantriols or pententriols, pentantetraol, 1,2,6-hexane triol and other hexane triols, hexane tetraol and -pentaol, heptane diol, -triol, -tetraol, -pentaol and -hexaol, 1,4-butane diol- diglycidyl-ether, etc. Short-chain, di-, tri-, tetra-, penta- and hexa-oxyethylene glycols and -ethylene glycols are also suitable for the present purpose as well as cyclic alcohols, such as benzyl alcohol, cyclopentanol, cyclohexanol, 3-, 4-, 5-cyclohexanol, cyclohexyl alcohol, aryl-alcohols, such as phenyl-ethanol, etc.

A nonionic edge active substance is any material which contains at least one, and in the majority of cases several, strongly hydrophilic groups and at least one, sometimes also several relatively hydrophobic, water insoluble residues. 'Nonionic' edge active substances can be zwitterionic or truly non-ionic.

$$R_1 - ((X_i - Y_i)_k - Z_1)_m - R_2 \quad (3)$$

The residues R₂ or X in such lipids often represent an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 8-24 carbon atoms. Very frequently, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, n-dodecyl, n-tetradecyl or n-tetradecenoyl, n-hexadecyl, n-

Sorbitol is one possible example of residue Z. $(X_i - Y_j)$ can be a polyene, polyoxyalkene, such as polyoxyethylene, polyalcohol, such as polyglycol, or polyether. $(X_i - Y_j)$ mainly contain 1-20 and very frequently 2-10 units, e.g. in ethylene glycol, di- and triglycol (oligoglycol) or polyethylene glycol.

Amongst the most common nonionic surfactants of the ether-type which are suitable for the present purpose are the substances of the Myrj trademark, such as polyoxyethylene(8)-stearate (Myrj45), polyoxyethylene(20)-stearate (Myrj49), polyoxy-

Another important special form of the nonionic edge active substance according to basic formula 3 most frequently contains a hydroxyl group in the position of residue R_1 and a hydrogen atom in the position of residue R_2 , by and large. Residues X and Z are frequently an alkoxy- or alkenoxy-, in principle also a hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 4-100 carbon atoms. Residue Y, too, is frequently an alkoxy-, alkenoxy-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain but one which is often branched and carries one methyl- or ethyl-side chain. Perhaps the most widely used edge active substances of this class are the surfactants which are marketed under the trademark "Pluronic".

Chains in the substances of TWEEN type are very frequently of

the polyoxyethylene class. They mainly contain one terminal hydrogen atom and more rarely a methoxy group. One of the polyoxyethylene chains, however, contains a hydrophobic residue which preferably corresponds to an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 4-24, and in particular 12-18 carbon atoms.

Edge active substances which are sold under the trademark "TRITON" are also useful according to this invention.

Polyalcohol residues R_2 are most frequently esterified or etherified; however, in some cases they can also be bound to the hydrophobic chain through a nitrogen atom. They are very often adducts of ethyleneglycol, glycerol, erythritol, or pentaerythritol, for example 1-alkyl-, 1-alkenyl-, 1-hydroxyalkene-glycerol, or corresponding 1,2-, or 1,3-diglycerides (for example, 1-alkyl,2-alkyl-, 1-alkyl,2-alkenyl-, 1-alkenyl,2-alkyl-, 1-alkenyl,2-alkenyl-, 1-alkenyl,2-hydroxyalkyl-, 1-hydroxyalkyl,2-alkenyl-, 1-alkyl,2-hydroxyalkyl-, 1-hydroxyalkyl,2-alkyl-, 1-alkenyl,2-hydroxyalkene-, 1-hydroxyalkene,3-alkenyl-, 1-alkyl,3-alkyl-, 1-alkyl,3-alkenyl-, 1-alkenyl,3-alkyl-, 1-alkenyl,3-alkenyl-, 1-alkenyl,3-hydroxyalkyl-, 1-hydroxyalkyl,3-alkenyl-, 1-alkyl,3-hydroxyalkyl-, 1-hydroxyalkyl,3-alkyl-, 1-alkenyl,3-hydroxyalkene- or 1-hydroxyalkene,3-alkenyl-). Glycerol can be replaced by another oligo- or polyalcohol, such as erythritol, pentantriol, hexantriol, -tetraol or -pentaol, etc., resulting in a wide variety of linkage possibilities.

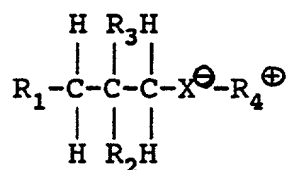
Z or R₂, moreover, can contain one or more 1-10, preferably 1-6, most frequently 1-3 carbohydrate residues or their derivatives. 'Carbohydrate residue' in this context has the meaning as already described and is an alpha or beta and L- or D-alloside, -altroside, -fucoside, -furanoside, -galactoside,

-galactopyranoside, -glucoside, -glucopyranoside, -lactopyranoside, -mannoside, -mannopyranoside, -psicoside, sorboside, -tagatoside, -taloside; frequently used derivatives of disaccharides are L- or D-maltopyranoside, -maltoside, -lactoside, malto- or -lactobionamide; the corresponding derivatives of maltotriose or -tetraose are also useful.

The carbohydrate residue can also contain a sulfur atom, e.g. in beta-L- or D-thiogluco-pyranoside or -thioglycoside.

Zwitterionic surfactants are substances, for example, which contain a sulphonate group, such as (3-((3-cholamidopropyl)-dimethylyammonio)-1-propanesulfonate (CHAPS) and (3-((3-cholamidopropyl)-dimethylyammonio)-2-hydroxy-1-propane-sulfonate (CHAPSO) or N-octyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfo-nate (lauryl-sulfobetaine), N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (myristyl-sulfobetaine), N-hexa-decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (palmityl-sulfobetaine), N-octadecyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate (stearyl-sulfobetaine), 'N-octadecenoyl-N,N,-dime-thyl-3-ammonio-1-propanesulfonate (oleoyl-sulfobetaine) etc.

Zwitterionic surfactants are also substances with the basic formula 4



in which n is one or zero. One of both side chains R₁ and R₂ contains one acyl-, alkyl-, alkenyl-, alkenoyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-, or alkoxy chain with 8-24 carbon atoms each; the other residue corresponds to a hydrogen, to a hydroxy group or to a short chain alkyl

residue. R_3 normally represents a hydrogen atom or a short alkyl chain. X is most frequently anionic, e.g. in a phosphate- or sulfate-residue. The residue R_4 in this case is cationic, in order to ensure that the whole molecule is zwitterionic. Most frequently, ammonio-alkyl derivatives, such as ethanol-, propanol-, butanol-, pentanolamine, hexanolamine, heptanolamine or octanolamine, N-methyl-, N,N-dimethyl, or N,N,N-trimethyl-ammonio-alkyl, N-ethyl-, N,N-diethyl, or N,N,N-triethyl-amino-alkyl, unequal N-alkyles, such as N,N-methyl-ethyl-ammonio-alkyl, or corresponding hydroxyalkyl substances are used, sometimes in a substituted form. (Single chain (lyso) derivatives of all biological zwitterionic phospholipids as well as their modified forms (such as Platelet-Activating-Factor and its analogs) also belong to this category.) R_4 can also be a positively charged carbohydrate residue, such as an aminosugar or one of its derivatives. R_4 and X, moreover, can exchange positions.

An ionic edge active substance is any material which contains at least one positive or negative charge and at least one segment which is poorly water soluble. An anionic substance of this kind can also contain several charges but must have a negative total charge. The total charge of any cationic substance must be positive.

Anionic edge active substances are for example the substances described by the basic formula 5:



in which R_1 is an organic hydrocarbon residue, which can also be substituted, and G^+ is a monovalent counterion, chiefly an alkali metal cation (such as lithium, sodium, potassium,

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rubidium, or cesium), an ammonium ion or a low weight tetraalkylammonium-ion, such as tetramethylammonium or tetraethylammonium.

The hydrocarbon residue R_1 in an anionic surfactant of the basic formula 5 is frequently a straight chain or branched acyl, alkyl or alkenoyl, or oxidized or hydroxygenated derivative thereof; the residue R_1 can also contain one or several cyclic segments.

R_1 chain frequently contains 6-24, more frequently 10-20, and most frequently 12-18 carbon atoms; if unsaturated, it contains 1-6, and even more frequently 1-3, double bonds in n-3- or n-6- position.

The following hydroxyalkyl chains are preferred for the present purpose: n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), n-octadecylhydroxy (hydroxystearyl), n-eicosylhydroxy or n-docosyloxy. Amongst the hydroxyacyl chains, the hydroxylauroyl, hydroxymyristoyl, hydroxypalmitoyl, hydroxystearoyl, eicosoylhydroxy or docosoyloxy chains are especially worth mentioning; particularly interesting amongst the hydroxyalkene-residues are the hydroxydodecen, hydroxytetradecen, hydroxyhexadecen, hydroxyoctadecen, hydroxyeicosen, hydroxydocosen, most notably 9-cis,12-hydroxy-octadecenyl (ricinolenyl) or 9-trans,12-hydroxy-octadecenyl (ricinelaidyl), 5-cis,8-cis,11-cis,14-cis,15-hydroxy-eicosatetraenyl (15-hydroxy-arachidonyl), 5-cis,8-cis,11-cis,14-cis,15-hydroxy,17-cis-eicosapentaenyl, 4-cis,7-cis,10-cis,13-cis,15-hydroxy,16-cis-docosapentaenyl and 4-cis,7-cis,10-cis,13-cis,15-hydroxy,16-cis,19-cis-docosahexaenyl.

Another class of anionic, edge active substances corresponds

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to basic formula 6



here, R_1 is a hydrocarbon residue which can also be substituted; X is a short-chain alkyl residue and Y denotes a sulfonate-, sulfate-, phosphate-, phosphonate or phosphinate group. G^+ is a mostly monovalent counterion (cation).

Alkali metal alkyl- or -alkenylethersulfonates or -phosphates belong to this class of ether-bonded molecules. Special examples are sodium- or potassium-n-dodecyloxyethylsulfate, -n-tetradecyloxyethylsulfate, -n-hexadecyl-oxyethylsulfate or -n-octadecyloxyethylsulfate or an alkali metal alkane sulfonate, such as sodium- or potassium-n-hexanesulfonate, n-octansulfonate, n-decansulfonate, n-dodecansulfonate, -n-tetradecansulfonate, -n-hexadecansulfonate or -n-octadecansulfonate.

The substances of general formula 7



are related to the compounds of basic type 6. These are analogous to the substances of formula 6 but contain a directly (covalently) coupled charged headgroup.

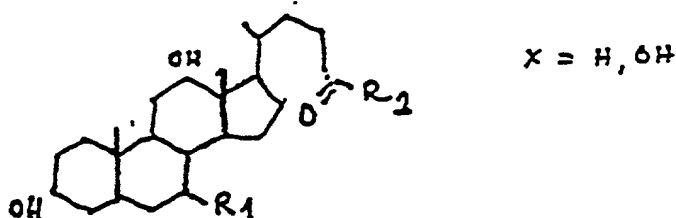
Particularly useful anionic, edge active substances of above formula 6 are alkali metal-alkylsulfates. To mention just a few examples: sodium or potassium-n-dodecyl (lauryl)-sulfate, -n-tetradecyl (myristyl)-sulfate, -n-hexadecyl (palmityl)-sulfate, -n-octadecyl (stearyl)-sulfate, n-hexadecylen (palmitolein)-sulfate and n-octadecylen (olein)-sulfate. Instead of a sulfate group, sulfonate, n-methyl- or n-ethylglycine for example can also be used.

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Various salts of bis-(2-alkyl-alkyl)-sulfosuccinate are also suitable for the applications as described in this work. Preferably, these are used as lithium-, sodium-, potassium-, or tetramethylammonium-bis-(2-ethyl-hexyl)-sulfosuccinate.

Furthermore, sarcosides, as well as alkyl- or alkenoyl-sulfochloride derivatives of the protein condensates, sulfonamide soaps, sulfatated or phosphorylated alcohol-esters, sulfatated or phosphorylated amides or monoglycerides, moreover, fatty acid alkylamides, sulfo- or phospho-succinic acid esters, taurides, alkylphenol-, alkylbenzol-, alkyl-naphthalene-ethersulfonates etc., are also all useful.

Another important group of anionic edge active substances are the derivatives of cholic acid. Their basic formula reads



here, R_1 corresponds to a proton, an OH - or a carbonyl group and R_2 can be a derivative of taurine or glycocoll, for example. Particularly suitable are various salts of cholic acid (bile acid, 3 α ,7 α ,12 α -trihydroxy-5 β -cholane-24-oin-acid), deoxycholic acid (3 α ,12 α -dihydroxy-5 β -cholane-24-oin-acid), chenodeoxycholic acid, glycocholic acid (N-(3 α ,7 α ,12 α -trihydroxy-24-oxycholane-24-yl-)glycine), deoxycholic acid, glycodeoxycholic acid (N-(3 α ,12 α -dihydroxy-24-oxycholane-24-yl-)glycine), glycochenodeoxycholic acid, glycolitocholic acid, glycoursodeoxycholic acid, lithocholic acid, taurodeoxycholic

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acid, taurocholic acid (3alpha,7alpha,12alpha-trihydroxy-5beta-cholan-24-oin-acid-N-(sulfoethyl)amide), taurochenodeoxycholic acid, tauroglycocholic acid, taurolitocholic acid, taurolitocholic acid-3-sulfate, tauroursodeoxycholic acid,ursocholanolic acid, ursodeoxycholic acid (3alpha,7beta-dihydroxy-5beta-cholanolic acid), the most common counterions being sodium or potassium.

Diverse cholic acid esters, such as cholesteryl-alkyl-, -alkenyl-, -hydroxyalkyl-, -hydroxyalkene-esters or cholesterylsulfates and -sulfonates are also edge active according to this invention.

Related synthetic adducts of the CHAPS class can also be used; in this case, R₂ is frequently an NH-(CH₂)₃-N',N'-(CH₂)₂(CH₂)₂-R₃-CH₂-SO₃ segment, whilst R₃ can be a proton or a carbonyl group. Again, sodium or potassium are the most commonly used counterions.

Digitonines as well as saponines, such as Quillaja acid, have similar basic structures in their cores as the cholic acid derivatives; consequently, they can also be used as edge active substances according to this invention.

The basic formula of the phosphorus-containing anionic edge active substances is



in which n is zero or one. One of the two side chains R₁ and R₂ contains hydrogen, a hydroxy group or a short chain alkyl residue; the other contains an alkyl-, alkenyl-, hydroxy-

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alkyl-, hydroxyalkenyl- or hydroxyacyl-chain (or an alkenyl-, alkoxy-, alkenyloxy- or acyloxy-residue) with 8-24 carbon atoms. The R_3 residue, as a rule, corresponds to hydrogen or an alkyl chain with less than 5 carbon atoms. R_4 can be an anionic oxygen or a hydroxy group; an alkyl chain with up to 8 C-atoms can also appear as well as another carbohydrate residue with up to 12 carbon atoms; if R_1 as well as R_2 are hydrogen and/or hydroxy groups, a steroid residue, a sugar derivative, a chain containing an amino group, etc., can also appear. Alkyl residues can also be substituted.

Amongst the most suitable surfactants of this substance class are: n-tetradecyl(=myristoyl)-glycero-phosphatidic acid, n-hexadecyl(=plamityl)-glycero-phosphatidic acid, n-octadecyl(=stearyl)-glycero-phosphatidic acid, n-hexadecylene(=palmitoleil)-glycero-phosphatidic acid, n-octadecylene(=oleil)-glycero-phosphatidic acid, n-tetradecyl-glycero,phosphoglycerol, n-hexadecyl-glycero-phosphoglycerol, n-octadecylene-glycero- phosphoglycerol, n-tetradecyl-glycero-phosphoserine, n-hexadecyl-glycerophosphoserine, -n-octadecyl-glycero-phosphoserine, n-hexadecylene-glycero-phosphoserine and n-octadecylene-glycero-phosphoserine.

The corresponding lyso-sulfolipids, phosphono- or phosphino-lipids are also suitable edge active compounds according to this invention.

Counterion in these compounds is most frequently an alkali metal cation (such as lithium, sodium, potassium, cesium) or a water soluble tetraalkylammonium-ion (such as tetramethyl-ammonium, tetrathylammonium, etc.).

All corresponding statements made above for surfactants of basic formula 3 also pertain to the carbohydrate residue R_1 .

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This residue in the majority of cases is a straight chain or branched alkyl or alkenoyl chain with 6-24, very frequently 10-20, in particular 12-18, carbon atoms and 1-6, especially frequently 1-3, double bonds in n-3- or n-6- positions.

Very convenient alkyl-residues R_1 or R_2 are, for example, n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, n-eicosyl or n-docosyl chains. N-nonyl, n-undecyl, n-tridecyl, n-pentadecyl, n-heptadecyl and n-nonadecyl, however, are equally useful.

An alkenyl in position R_1 or R_2 is preferably a 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleoyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl), 11-cis-octadecenyl (vaccenyl), 9-cis-eicosenyl (gadoleinyl), 13-cis-docosenyl, 13-trans-docosenyl or 15-cis-tetracosenyl, etc.

Higher unsaturated alkenyls which also can be used for the present purpose are, amongst others: 9-cis,12-cis-octadecendienyl, 9-trans,12-trans-octadecendienyl, 9-cis,12-cis,15-cis-octadecentrienyl, 6-cis,9-cis,12-cis-octadecentrienyl, 11-cis,14-cis,17-cis-eicosatrienyl, 6-cis,9-cis,12-cis,15-cis-octadecentetraenyl, 5-cis,8-cis,11-cis,14-cis-eicosatetraenyl, 5-cis,8-cis,11-cis,14-cis,17-cis-eicosapentaenyl, 4-cis,7-cis,10-cis,13-cis,16-cis-docosapentaenyl and 4-cis,7-cis,10-cis,13-cis,16-cis,19-cis-docosahexaenyl.

R_1 and R_2 are preferably chosen from the substances of the hydroxyalkyl-class, in which case they correspond, for example, to n-decylhydroxy, n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), n-octadecylhydroxy (hydroxystearyl) and n-

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eicosylhydroxy (hydroxyarachinyl) chains.

An alkenylhydroxy in R_1 or R_2 is preferably a 9-cis-dodecenylhydroxy (hydroxylauroleyl), 9-cis-tetradecenylhydroxy (hydroxymyristoleyl), 9-cis-hexadecenylhydroxy (hydroxypalmitoleinyl), 6-cis-octadecenylhydroxy (petroselinylhydroxy), 6-trans-octadecenylhydroxy (hydroxypetroselaidinyl), 9-cis-octadecenylhydroxy (hydroxyoleyl), 9-trans-octadecenylhydroxy (hydroxyelaidinyl) and 9-cis-eicosenyl (hydroxygadoleinyl) chain.

An alkanoylhydroxy in R_1 or R_2 is preferably an n-decanoylhydroxy, n-dodecanoylhydroxy (lauroylhydroxy), n-tetradecanoylhydroxy (myristoylhydroxy), n-hexadecanoylhydroxy, n-hexadecanoylhydroxy (palmitoylhydroxy), n-octadecanoylhydroxy (stearoylhydroxy) and n-eicosoylhydroxy (arachinoylhydroxy) chain.

An alkenoylhydroxy in R_1 or R_2 is preferably a 9-cis-dodecenylhydroxy (lauroleoylhydroxy), 9-cis-tetradecenoylhydroxy (myristoleoylhydroxy), 9-cis-hexadecenoylhydroxy (palmitoleinoylhydroxy), 6-cis-octadecenoylhydroxy (petroselinoylhydroxy), 6-trans-octadecenoylhydroxy (petroselaidinoylhydroxy), 9-cis-octadecenoylhydroxy (oleoylhydroxy), 9-trans-octadecenoylhydroxy (elaidinoylhydroxy) and 9-cis-eicosenoyl (gadoleinoylhydroxy) chain.

Some examples for the short chain alkyl residue, which often appear in the R_4 residue, are methylene-, ethylene-, n-propylene-, iso-propylene-, n-butylene- or iso-butylene- as well as n-pentylene- or n-hexylene-groups. R_4 can also be a carboxy- or a sulfo-group, an acid or alkaline group, such as carboxy- and amino-group; the amino group in such case is always in the alpha-position relative to the carboxy group.

Another example for the R_4 residue are free or etherified hydroxy groups (two ether-bonded hydroxy groups, in such case, can be connected by one divalent hydrocarbon residue, such as methylene, ethylene, ethylidene, 1,2-propylene or 2,2-propylene). R_4 , furthermore, can be substituted by a halogen atom, such as chlorine or bromine, a low weight alkoxy-carbonyl, such as methoxy- or ethoxycarbonyl, or by a low weight alkansulfonyl-, such as methansulfonyl.

A substituted short chain alkyl residue R_4 with 1-7 C-atoms is preferably carboxy-short-chain alkyl, such as carboxy-methyl, carboxyethyl- or 3-carboxy-n-propyl, omega-amino-n-carboxy- a short-chain alkyl, such as 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-short-chain alkyl, such as 2-hydroxyethyl or 2,3-dihydroxypropyl, a short-chain alkoxy-3-methoxy-n-propyl, a short-chain alkylendioxy-short-chain alkyl, such as 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)-dioxypropyl, or halogen-short-chain alkyl, such as chloro- or bromo-methyl, 2-chloro- or 2-bromo-ethyl, 2- or 3-chloro- or 2-or 3-bromo-n-propyl.

A carbohydrate residue R_4 with 5-12 C-atoms is, for example, a natural monosaccharide residue stemming from a pentose or a hexose in the aldose or ketose form.

A carbohydrate residue R_4 , moreover, can be a natural disaccharide residue, such as a disaccharide residue formed from two hexoses, in the described sense. A carbohydrate residue R_4 can also be a derivatised mono-, di- or oligosaccharide residue, in which an aldehyde group and/or one or two terminal hydroxy groups are oxidized to a carboxy group, e.g. a D-glucon-, D-glucar- or D-glucoron acid residue; this preferably appears in the form of a cyclic lactone residue. The aldehyde- or keto-groups in a derivatised mono-

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or disaccharide residue can also be reduced to a hydroxy group, e.g. in inositol, sorbitol or D-mannitol; also, one or several hydroxy groups can be replaced by a hydrogen atom, e.g. in desoxysugars, such as 2-desoxy-D-ribose, L-rhamnose or L-fucose, or by an amino group, e.g. in aminosugars, such as D-glucosamine or D-galactosamine.

R_4 can also be a steroid residue or a sterine residue. If R_4 is a steroid residue, R_3 is a hydrogen atom, whilst R_1 and R_2 in such case preferably correspond to a hydroxy group.

The counterion in such cases is preferably an ammonium, sodium or potassium ion.

In an anionic surfactant of formula 8, the following values of parameters are preferred: $n = 1$, R_1 is an alkyl, such as n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl or n-octadecyl (stearyl), hydroxyalkyl, such as n-dodecylhydroxy (hydroxy-lauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), or n-octadecylhydroxy (hydroxystearyl), hydroxyacyl, such as hydroxylauroyl, hydroxymyristoyl, hydroxypalmitoyl or hydroxystearoyl, R_2 is a hydrogen atom or a hydroxy group, R_3 is a hydrogen atom or a short-chain alkyl, such as methyl, R_4 is a short-chain alkyl, e.g. methyl or ethyl, short-chain alkyl substituted by an acid or an alkaline group, such as a carboxy and amino group, e.g. omega-amino-omega-carboxy-short-chain alkyl, such as 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-short-chain alkyl, such as 2-hydroxyethyl or 2,3-hydroxypropyl, short-chain alkylenedioxy-short-chain alkyl, e.g. 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)-dioxypropyl, halogen-short-chain alkyl, such as 2-chloro- or 2-bromo-ethyl group, a carbohydrate residue with 5-12 C-

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atoms, e.g. in inositol, or a steroid residue, such as a sterol, e.g. cholesterol, and G^+ is a sodium-, potassium- or ammonium-ion.

An anionic surfactant of formula 8, in many cases, is a sodium- or potassium salt of lysophosphatidylserine, such as the sodium- or potassium salt of lysophosphatidylserine from bovine brain or the sodium- or potassium salt of a synthetic lysophosphatidylserine, such as sodium- or potassium-1-myristoyl- or -1-palmitoyl-lysophosphatidylserine, or a sodium- or potassium salt of lysophosphatidylglycerols. The hydrogen atom on the phosphate group can be replaced by a second cation, G^+ or calcium-, magnesium-, manganese-ion, etc.

An anionic surfactant of formula 8 preferably contains an alkyl chain, such as n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristoyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl or n-octadecyl (stearyl), a hydroxyalkyl chain, such as n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), or n-octadecylhydroxy (hydroxystearyl), a hydroxyacyl chain, such as hydroxylauroyl, hydroxymyristoyl, hydroxypalmitoyl or hydroxystearoyl in position R_1 , a hydrogen atom or a hydroxy group in position R_2 , and a hydrogen atom or a short-chain alkyl, such as methyl group, in position R_3 . G^+ is preferably an ammonium, sodium, potassium or tetramethylammonium ion.

An anionic surfactant of formula 8 can, furthermore, be a sodium- or potassium salt of a natural phosphatidic acid, such as egg-phosphatidic acid, a sodium- or potassium salt of a natural lysophosphatidic acid, such as egg-lysophosphatidic acid, a sodium- or potassium salt of a synthetic lysophosphatidic acid, such as 1-lauroyl-, 1-myristoyl-, 1-palmitoyl- or 1-oleoyl-lysophosphatidic acid, etc.

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The most important classes of cationic surfactants encompass: ammonium salts, quarternary ammonium salts, salts of heterocyclic bases, such as alkylpyridium-, imidazole-, or imidazolinium salts, salts of alkylamides and polyamines, salts of acylated diamines and polyamines, salts of acylated alkanolamines, salts of alkanolamine esters and ethers, etc.

A cationic surfactant is, for example, any substance corresponding to the formula:



in which R_1 is a hydrocarbon residue which can also be substituted. R_2 denotes a short-chain alkyl, phenyl-short-chain-alkyl or hydrogen atom. R_3 and R_4 correspond to a short-chain alkyl residue. R_2 and R_3 , together with the nitrogen atom, represent an aliphatic heterocycle, which can also be substituted on a carbon atom; R_4 is a short-chain alkyl; R_2 , R_3 and R_4 , together with the nitrogen atom, can also form an aromatic heterocycle, which, moreover, can be substituted on one of the carbon atoms. G^- corresponds to an anion.

In a cationic surfactant of basic formula 9, R_1 represents an aliphatic hydrocarbon residue, which can also be substituted, for example, by an aryloxy- short-chain-alkoxy-, a substituted short-chain alkyl, a straight chain or branched chain alkyl with 7-22, and in particular 12-20, carbon atoms, or an alkenyl with 8-20, or in particular 12-20, carbon atoms and 1-4 double bonds.

An alkenyl with 8-24, in particular 12-22, carbon atoms and 0-5, in particular 1-3, double bonds is e.g. 1-octenyl, 1-nonenyl, 1-decenyl, 1-undecenyl, 1-dodecenyl, 9- cis-dodecenyl (lauroleyl), 1-tridecenyl, 1-tetradecenyl, 9-cis-tetradecenyl (myristoleyl), 1-pentadecenyl, 1-hexadecenyl, 9-cis-hexadecenyl (palmitoleinyl), 1-heptadecenyl, 1-octadecenyl, 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl), 9-cis-12-cis-octadecadienyl (linoleyl), 9-cis-11-trans-13-trans-octadecatrienyl (alpha-elaostearinyl), 9-trans-11-trans-13-trans-octadecatrienyl (beta-elaostearinyl), 9-cis-12-15-cis-octadecatrienyl (linolenyl), 9-, 11-, 13-, 15-octadecatetraenyl (parinaryl), 1-nonadecenyl, 1-eicosenyl, 9-cis-eicosenyl (gadoleinyl), 5-, 11-, 14-eicosatrienyl or 5-, 8-, 11-, 14-eicosatetraenyl (arachidonyl).

Methyl or ethyl are two examples of short-chain alkyl residues R_2 , R_3 or R_4 which appear in substances of formula 9.

Two examples of phenyl-short-chain-alkyl groups in R_2 are benzyl or 2-phenylethyl.

Substituents of this heterocycle are the substituents R_1 and R_4 on the nitrogen as well as, in some cases, on the carbon atom; they are, most frequently, of the short-chain alkyl, such as methyl, ethyl, n-propyl or n-butyl type.

An aromatic heterocycle, formed from R_2 , R_3 and R_4 together with the nitrogen atom, is, for example, a monocyclic five- or six-member aza-, diaza-, oxaaza- or thiazacyclic residue, such as pyridinio, imidazolinio, oxazolinio or thiazolinio or, for example, a benzocondensed monoazabicyclic residue, such as chinolinio or iso-chinolinio group.

A heterocycle, formed from R₂, R₃ and R₄ and substituted on a carbon atom through the mentioned residues is, for example, a 2- or 4-short-chain-alkylpyridinio, e.g. 2- or 4-methyl or 2- or 4-ethylpyridinio, di-short-chain-alkylpyridinio, e.g. 2,6-

dimethyl-, 2-methyl-3-ethyl-, 2-methyl-4-ethyl-, 2-methyl-5-ethyl-, or 2-methyl-6-ethylpyridinio, 2-, 3- or 4-halogenpyridinio, e.g. 2-, 3- or 4-chloropyridinio or 2-, 3- or 4-bromo-pyridinio, 2-short-chain alkylimidazolinio, -oxazolinio or -thiazolinio, such as 2-methyl- or 2-ethylimidazolinio, -oxazolinio or -thiazolinio or 2-short-chain alkyl-8-halogenchinolinio, such as 2-methyl-8-chlorochinolinio group.

A cationic surfactant of formula 9 is preferably an N-benzyl-N,N-dimethyl-N-2-(2-(4-(1,1,3,3-tetramethylbutyl)-phenhydroxy)-ethhydroxy)-ethylammoniochloride, N-benzyl-N,N-dimethyl-N-2-(2-(3(methyl-4-(1,1,3,3-tetramethylbutyl)-phenhydroxy)-ethhydroxy)-ethylammoniochloride (methylbenzethoniumchloride), n-dodecyltrimethylammoniochloride or -bromide, trimethyl-n-tetradecylammoniochloride or -bromide, n-hexadecyltrimethylammoniochloride or -bromide (cetyltrimethyl-ammoniumchloride or -bromide), trimethyl-n-octadecylammoniochloride or -bromide, ethyl-n-dodecyl-dimethylammoniochloride or -bromide, ethyldimethyl-n-tetradecylammoniochloride or -bromide, ethyl-n-hexadecyldimethylammoniochloride or -bromide, ethyldimethyl-n-octadecylammoniochloride or -bromide, n-alkyl-benzyl-dimethyl-ammoniochloride or -bromid (benzalkoniumchloride or -bromide), such as benzyl-n-dodecyldimethylammoniochloride or bromide, benzyldimethyl-n-tetradecylammoniochloride or -bromide, benzyl-n-hexadecyldimethyl-ammoniochloride or -bromide or benzyldimethyl-n-octadecylammonio-chloride or -bromide, N-(n-decyl)-pyridiniochloride or -bromide, N-(n-dodecyl)-pyridiniochloride or -bromide, N-(n-tetradeyl)-pyridiniochloride or -bromide, N-(n-hexadecyl)-pyridiniochloride or -bromide (cetylpyridiniumchloride) or N-(n-octadecyl)-pyridinio-chloride or -bromide. Mixtures of these or other edge active substances are also suitable.

The following surfactants are especially useful for biological

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purposes: N,N-bis(3-D-glucon-amidopropyl)cholamide (BigCHAP), Bis(2-ethylhexyl)sodium-sulfosuccinate, cetyl-trimethyl-ammonium-bromide, 3-((cholamidopropyl)-dimethylammonio)-2-hydroxy-1-propane sulfonate (CHAPSO), 3-((cholamidopropyl)-dimethylammonio)-1-propane sulfonate (CHAPS), cholate-sodium salt, decaoxyethylene-dodecyl-ether (Genapol C-100), decaethylene-isotridecyl-ether (Genapol X-100), decanoyl-N-methyl-glucamide (MEGA-10), decyl-glucoside, decyl-maltoside, 3-(decyldimethylammonio)-propane-sulfonate (Zwittergent 3-10), deoxy-bigCHAP, deoxycholate, sodium salt, digitonin, 3-(dodecyldimethylammonio)-propane-sulfonate (Zwittergent 3-12), dodecyl-dimethyl-amine-oxide (EMPIGEN), dodecyl-maltoside, dodecylsulfate, glyco-cholate, sodium salt, glyco-deoxycholate, sodium salt, heptaethylene-glycol-octyl-phenyl-ether (triton X-114), heptyl-glucoside, heptyl-thiogluco-side, 3-(hexadecyldimethylammonio)-propane-sulfonate (Zwittergent 3-14), hexyl-glucoside, dodecyl-dimethyl-amine-oxide (Genaminox KC), N-dodecyl-N,N-dimethylglycine (Empigen BB), N-decyl-sulfobetaine (Zwittergent 3-10), N-dodecyl-sulfobetaine (Zwittergent 3-12), N-hexadecyl-sulfobetaine (Zwittergent 3-16), N-tetradecyl-sulfobetaine (Zwittergent 3-14), N-octyl-sulfobetaine (Zwittergent 3-08), nonaethylene-glycol-mono-dodecyl-ether (THESIT), nonaethylene-glycol-octyl-phenol-ether (triton X-100), nonaethylene-glycol-octyl-phenyl-ether (NP-40, Nonidet P-40), nonaethylene-dodecyl-ether, nonanoyl-N-methyl-glucamide (MEGA-9), nonaoxyethylene-dodecyl-ether (Lubrol PX, Thesit), nonyl-glucoside, octaethylene-glycol-isotridecyl-ether (Genapol X-080), octaethylene-dodecyl-ether, octanoyl-N-methyl-glucamide (MEGA-8), 3-(octyldimethylammonio)-propane-sulfonate (Zwittergent 3-08), octyl-glucoside, octyl-thiogluco-side, entadecaethylene-isotridecyl-ether (Genapol X-150), polyethylene-polypropylene-glycol (Pluronic F-127), polyoxyethylene-sorbitane-monolaurate (Tween 20), polyoxy-ethylene-sorbitane-monooleate (Tween 80), taurodeoxycholate-sodium salt, taurocholate-sodium salt, 3-(tetradecyl-

dimethylammonio)-propane-sulfonate (Zwittergent 3-14), etc.

Particularly suitable for pharmacological purposes are:
cetyl-trimethyl-ammonium-salts (such as hexadecyltrimethylammoniumbromide, trimethylhexadecylamine-bromo-salt), cetylsulfate salts (such as Na-salt, Lanette E), cholate salts (such as Na- and ammonium-form) decaoxyethylene-dodecyl-ether (Genapol C-100), deoxycholate salts, dodecyl-dimethyl-amine-oxide (Genaminox KC, EMPIGEN), N-dodecyl-N,N-dimethylglycine (Empigen BB), 3-(hexadecyldimethylammonio)-propane-sulfonate (Zwittergent 3-14), fatty acid salts and fatty alcohols, glyco-deoxycholate salts, laurylsulfate salts (sodium dodecylsulfate, Duponol C, SDS, Texapon K12), N-hexadecyl-sulfobetaine (Zwittergent 3-16), nonaethylene-glycol-octyl-phenyl-ether (NP-40, Nonidet P-40), nonaethylene-dodecyl-ether, octaethylene-glycol-isotridecyl-ether (Genapol X-080), octaethylene-dodecyl-ether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monostearate (Tween 60), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-cetylstearylether (Cetomacrogol, Cremophor O, Eumulgin, C 1000) polyhydroxyethylene-4-laurylether (Brij 30), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrogenated castor oil (Cremophor RH 40, Cremophor RH 60) polyethoxylated plant oils (Lebrafils), sorbitane-monolaurate (Arlacel 20, Span 20), taurodeoxycholate salts, taurocholate salts, polyethylene glycol-20-sorbitane-palmitate (Tween 40), Myrj 49 and polyethylene glycol derivatives of ricinols, etc.

AGENTS:

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Transfersomes as described in this invention are suitable for the application of many different agents and, in particular, for therapeutic purposes, for example. The preparations according to this invention can contain the following:

- at least one adrenocorticostatic agent, in particular metyrapon;
- at least one carrier substance, additive or agent, belonging to the class of beta-adrenolytics (beta blocking agents), very frequently acetobol, alprenolol, bisoprololfumarate, bupranolol, carazolol, celiprolol, mepindolsulfate, metipranolol, metoprolotartat, nadolol, oxyprenolol, pindolol, sotalol, tertatolol, timolohydrogen maleate and toliprolol, especially preferred, atenolol or propranolol;
- at least one carrier substance, additive or agent, belonging to the androgenes or antiandrogenes, in particular drostanolonpropionate, mesterolone, testosteroneundecanoate, testolacton, yohimbine, or chloroamidinonacetate, cyproteronacetate, ethinylestradiol or flutamide;
- at least one carrier substance, additive or agent with an antiparasitic action, frequently phanquinone, benzyobenzoate, bephenium-hydroxy-naphthoate, crotamitone, diethylcarbamazine, levamisol, lindane, malathione, mesulfene (2,7-dimethylantren), metronidazol or tetramisol;
- at least one anabolic agent, in particular clostebolacetate, cyanocobolamine, folic acid, mestanolone, metandienone, metenolone, nandrolone, nandrolondecanoate, nandrolone-hexyloxyphenylpropionate,

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nandrolon-phenyl-propionate, norethandrolone,
oxaboloncipionate, piridoxine or stanozolole;

- at least one agent which can induce systemic anesthesia or analgesia, e.g. chlorobutanol, ketamine, oxetacaine, propanidide and thiamylal, aminophenol-derivatives, aminophenazol-derivatives, antranilic acid- and arylpropione acid derivatives, azapropazone, bumadizone, chloroquin- and codeine-derivatives, diclophenac, fentanil, ibuprofen, indometacine, ketoprofen, methadone-substances, morazone, morphine and its derivatives, nifenazone, niflumin acid, pentazozine, pethidine, phenazopyridine, phenylbutazone-derivatives (such as 3,5 pyrazolidine dion), pherazone, piroxicam, propoxyphene, propyphenazon, pyrazol- and phenazone-derivatives (aminophenazone, metamizole, monophenylbutazone, oxyphenebutazone, phenylbutazone or phenazone-salyzilate), salicylic acid-derivatives, sulfasalazine, tilidine; acetylsalicylic acid, ethylmorphine, alclofenac, alphaprodine, aminophenazone, anileridine, azapropazone, benfotiamine, benorilate, benzydamine, cetobemidone, chlorophenesincarbamate, chlorothenoxazine, codeine, dextromoramide, dextro-propoxyphene, ethoheptazine, fentanyl, fenylramidol, fursultiamine, flupirtinmaleate, glafenine, hydromorphone, lactylphenetidine, levorphanol, mefenamic acid, meptazonol, methadone, mofebutazone, nalbufine, Na-salt of noramidopyrinium-methanesulfonate, nefopam, normethadone, oxycodone, paracetamol, pentazocine, pethidine, phenacetine, phenazocine, phenoperidine, pholcodine, piperylone, piritramide, procaine, propyphenazone, salicylamide, thebacone, tiemonium-odide, tramadone;
- at least one substance from the class of analeptics, such

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as aminophenazole, bemegride, caffeine, doxapram, ephedrine, prolintane, or nialamide and tranylcypromine; but also vitamins, plant extracts from semen colae, camphor, menthol;

- at least one substance from the class of antiallergics: e.g. agents from the globuline family, corticoids or antihistaminics (such as beclometasone-, betametasone-cortisone-, dexametasone-derivatives, etc.) as well as bamipinacetate, buclizine, clemastine, clemizole, cromoglicinic acid, cyproheptadine, diflucorolonvalerate, dimetotiazine, diphenhydramine, diphenylpyraline, ephedrine, fluocinolane, histapyrrodine, isothipendyle, methadilazine, oxomemazine, paramethasone, prednilidene, theophilline, tolpropamine tritoqualine, etc. are used; amongst the preferred agents in this class are the substances characterized by their capacity to interfere (stimulate or suppress) the production of immunologically active substances, such as interleukines, interferones, leucotrienes, prostaglandines, etc. Amongst others, certain lipids and lipoids, such as phosphatidylcholines and diacylglycerols, or fatty acids and their esters, with chains containing several, preferably 3-6, most very frequently 3 or 4, double bonds, preferably of the n-3 type, are used for this purpose; the latter may also be hydroxygenated, branched or (partially) derivatized into ring structures.
- at least one substance with antiarrhythmic action, such as most of the cardiacs and beta-blockers, ajmaline, bupranolol, chinidine, digoxine derivatives, diltiazem, disopyramidedihydrogensulfate, erythromycine, disopyramide, gallopamil, ipratropiumbromide, lanatoside, lidocaine, lorcainide, orciprenalinesulfate, procaine amide, propafenone, sparteinesulfate, verapamil,

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toliprolol.

- an antiarteriosclerotic, such as clofibrate.
- at least one substance belonging to the antiasthmatics and/or bronchospasmolytics, such as amiodarone, carbutole, fenoterol, orciprenaline, sotalol, or theophylline-derivatives, as well as corticoids (such as beclomethasone, dexamethasone, hydrocortisone, prednisolone), frequently in combination with purines;
- at least one substance from the class of antibiotics, such as actinomycine, alamethicine, alexidine, 6-aminopenicillanic acid, moxycillin, amphotericine, ampicillin, anisomycine, antiamoebine, antimycine, aphidicoline, azidamfenicol, azidocillin, bacitracine, beclomethasone, benzathine, benzylpenicillin, bleomycine, bleomycine sulfate, calcium ionophore A23187, capreomycine, carbenicillin, cefacetyl, cefaclor, cefamandole nafate, cefazolin, cefalexine, cefaloglycine, cefaloridine, cefalotidine, cefapirine, cefazolin, cefoperazone, ceftriaxone, cefuroxime, cephalaxine, cephaloglycine, cephalothine, cephalapirine, cerulenine, chloroamphenicol, chlorotetracycline, chloroamphenicol diacetate, ciprofloxacin, clindamycine, chloromadinone acetate, chlorpheniramine, chromomycine A3, cinnarizine, ciprofloxacin, clotrimazole, cloxacillin, colistin methanesulfonate, cycloserine, deacetylanisomycine, demeclocycline, 4,4'-diaminodiphenyl sulfone, diazepam, dicloxacillin, dihydrostreptomycine, dipyridamol, doxorubicine, doxycycline, epicillin, erythromycine, erythromycine-stolate, erythromycinethylsuccinate, erythromycine stearate, ethambutol, flucloxacillin, fluocinolone acetonide, 5-fluorocytosine, filipine, formycine,

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fumaramidomycine, furaltadone, fusidic acid, geneticine, gentamycine, gentamycine sulfate, gliotoxine, gfamicidine, griseofulvine, helvolic acid, hemolysine, hetacillin, kasugamycine, kanamycine (A), lasalocide, lincomycine, magnesidine, melphalane, metacycline, meticilline, mevinoline, micamycine, mithramycine, mithramycine A, mithramycine complex, mitomycine, minocycline, mycophenolic acid, myxothiazol, natamycine, nafcilline, neomycine, neomycine sulfate, 5-nitro-2-furaldehydesemicarbazone, novobiocine, nystatine, oleandomycine, oleandomycine phosphate, oxacihine, oxytetracycline, paromomycine, penicilline, pecilocine, pheneticilline, phenoxymethylpenicilline, phenyl amino-salicylate, phleomycine, pivampicilline, polymyxine B, propicilline, puromycine, puromycine aminonucleoside, puromycine aminonucleoside 5'-monophosphate, pyridinol carbamate, rolitetracycline, rifampicine, rifamycine B, rifamycine SV, spectinomycine, spiramycine, streptomycine, streptomycine sulfate, sulfabenzamide, sulfadimethoxine, sulfamethizol, sulfamethoxazol, tetracycline, thiamphenicol, tobramycine, troleandomycine, tunicamycine, tunicamycine A1-homologs, tunicamycine A2-homolog, valinomycine, vancomycine, vineomycine A1, virginiamycine M1, viomycine, xylostasine;

- at least one substance with an antidepressive or antipsychotic action, such as diverse monoaminoxidase-suppressors, tri- and tetracyclic antidepressives, etc. Very frequently used agents of this class are alprazolame, amitriptyline, chloropromazine, clomipramine, desipramine, dibenzepine, dimetacrine, dosulepine, doxepine, fluvoxaminhydrogenmaleate, imipramine, isocarboxazide, lofepramine, maprotiline, melitracene, mianserine, nialamide, noxiptiline,

nomifensine, nortriptyline, opipramol, oxypertine, oxytriptane, phenelzine, protriptyline, sulpiride, tranylcypromine, trosadone, tryptophane, vitoxazine, etc.

- at least one antidiabetic agent, such as acetohexamide, buformine, carbutamide, chloropropamide, glibenclamide, glibornuride, glymidine, metformine, phenformine, tolazamide, tolbutamide;
- at least one substance acting as an antidote, for example, against the heavy metal poisoning, poisoning with insecticides, against drugs, blood poisons, etc. A few examples are different chelators, amiphenazol obidoxim-chloride, D-penicillamine, tiopromine, etc.;
- at least one substance from the class of antiemetics: some of such suitable agents are alizapride, benzquinamide, betahistidine-derivatives, cyclizine, difenidol, dimenhydrinate, haloperidol, meclozine, metoclopramide, metopimazine, oxypendyl, perphenazine, pipamazine, piprinhydrinate, prochloroperazine, promazine, scopolamine, sulpiride, thiethylperazine, thioproperazine, triflupromazine, trimethobenzamide, etc., which are frequently used in combination with vitamins and/or antiallergics;
- at least one substance with an antiepileptic action, such as barbexaclone, barbiturate, beclamide, carbamazepine, chloroalhydrate, clonazepam, diazepam, ethosuximide, ethylphenacemide, lorazepam, mephénytoine, mesuximide, oxazolidine, phenaglycodol, phensuximide, phenytoine, primidone, succinimide-derivatives, sultiam, trimethadione, yalproinic acid, etc.; additives are commonly chosen from the classes of hypnotics and sedatives; an especially commonly used agent of this kind is

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[illegible]

- at least one substance belonging to the class of antihypertonics, such as many alpha-receptor agonists, aldosterone-antagonists, angiotensine-converting-enzyme-blockers, antisympathicotonics, beta-blockers, calcium-antagonists, diuretics, vasodilators, etc.; suitable agents for this purpose are for example alpenolol, atenolol, bendroflumethiazide, betanidine, butizide, chlorotalidone, clonidine, cycletanine, cyclopenthiazide, debrisoquine, diazoxide, dihydralazine, dihydroergo-taminmethanesulfonate, doxazinmesilate, guanethidine, guanoclor, guanoxane, hexamethonium-chloride, hydralazine, labetalol, mecanylanine, methyldopa, pargyline, phenoxybenzamine, prazosine, quinethazone, spironolactone, bescinnamine, reserpine, trichloro-methiazide or vincamine;
- at least one substance which is an inhibitor of biological activity, such as actinomycine C1, alpha-

Appendix

- at least one substance acting as an antihypotonic agent; quite frequently the corresponding drugs are from the classes of analeptics, cardiacs or corticoids. Suitable agents for this purpose are, for example, angiotensine-amide, cardaminol, dobutamine, dopamine, etifelmine,

etilefrine, gepefrine, heptaminol, midodrine, oxedrine, etc., especially norfenefrine;

- at least one substance from the group of anticoagulants. Among other substances, some coumarin-derivatives are suitable for this purpose, as well as heparine and heparinoids, hirudine and related substances, dermatan-sulfate etc.; most frequently used agents of this class are acenocoumarin, anisindione, diphenadione, ethyl-biscoumacetate, heparine, hirudine, phenprocoumon, as well as warfarine;
- at least one substance from the class of antimycotics; well-suited examples of such agents include: amphotericine, bifanozol, buclosamide, chinoline-sulfate chloromidazol, chlorophenesine, chloroquinaldol, clodantoine, cloxiquine, cyclopiroloxamine, dequaliniumchloride, dimazol, fenticlor, flucytosine, griseofulvine, ketoconazol, miconazol, natamycine, sulbentine, tioconazol, tolnaftate, etc.; particularly frequently, amphotericine, clotrimazol or nystatine are likely to be used for this purpose;
- at least one substance from the class of antimyasthenics, such as pyridostigmine-bromide;
- at least one substance which is active against morbus parkinson, such as amantadine, benserazide, benzatropine, biperidene, cycrimine, levodopa, metixene, orphenadrine, phenglutarimide, pridinol, procyclidine, profenamine or trihexyphenidyl;
- at least one substance with an antiphlogistic activity, such as aescine, acetylsalicylic acid, alclofenac, aminophenazone, azapropazone, benzydamine, bumadizone,

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chlorothenoxazine, diclofenac, flufenaminic acid, glafenine, ibuprofene, indometacine, kebuzone, mefenam acid, metiazic acid, mesalazine, mofebutazone, naproxene, niflumine acid, salts, such as Na-salt, noramido-pyriminium-methane-sulfonate, orgoteine, oxyphenbutazone, phenylbutazone, propyphenazone, pyridoxine, tolmetine, etc.; very suitable is, for example, ibuprofen; some of the agents commonly used as antiphlogistics also exhibit an antihistaminic or analgetic activity and belong to the classes of corticoids, vasoactiva, opthalmics or otologics;

- at least one substance which is an antipyretic, such as acetylsalicylic acid, alclofenac, aminophenazone, benzydamine, bumadizone, chinine, chlorinethenoxazine, lactylphenetidine, meprob, paracetamol, phenacetine, propyphenazone or salicylamide;
- at least one substance with an antirheumatic activity, such as acetylsalicylic acid, benorilate, chloroquine, diclofenac, fenoprofene, flufenaminic acid, ibuprofene, kebuzone, lactylphenetidine, mefenamic acid, mofebutazone, naproxene, sodiumaurothiomalate, nifenazone, nifluminic acid, D-penicillamine and salicylamide. Edge active substances, carriers and/or agents, with a hypoallergic action, for example from the groups of analgetics, corticoids and glucocorticoids, enzymes or vitamins, etc., are preferred for this purpose, as well as antiphlogistics, such as quinine, nicotinic acid-, nonylic acid-, or salicylic acid-derivatives, meprobamate, etc.;
- at least one antiseptic such as acriflaviniumchloride, cetalkonium-chloride, cetylpyridinium-chloride, chlorohexidine, chloroquinaldol, dequaliniumchloride,

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domiphen-bromide, ethacridine, hexetidine, merbromine, nitrofurazone, oxyquinol, phanquinone, phenazopyridine or phenylmercuriborate, as well as fatty acids with an uneven number of carbon atoms;

- at least one respiratory analeptic or respiration stimulant, such as amiphenazol, ascorbic acid, caffeine, cropropamide, crotethamide, etamivane, ephedrine, fominobene, nicethamide; or aminophenazol and doxapram, for example;
- at least one broncholytic, such as bamifylline, beclometasone, dexometasone (e.g. in dexometasone-21-isonicotinate), diprophylline, ephedrine (e.g. in ephedrinehydrogentartrate), fenoterol, hexoprenaline, ipratropium-bromide, iso-eterine, isoprenaline, orciprenaline, procylol, proxyphylline, reproterol, salbutamol, terbutaline, tetroquinol, theophylline, etc.; and biological extracts, for example from anis, eucalyptus, thyme, etc.;
- one cardiostimulant, especially aminophylline, benfurodilhemisuccinate, etofylline, heptaminol, protheobromine or proxyphylline;
- at least one substance from the class of chemotherapeutic agents, for example, acediasulfone, acriflavinium-chloride, ambazone, dapson, dibrompropamide, furazolidone, hydroxymethylnitrofurantoin, idoxuridine, mafenide and sulfateolamide, mepacrine, metronidazole, nalidixic acid, nifuratel, nifuroxazide, nifurazone, nifurtimox, ninorazole, nitrofurantoin, oxolinic acid, pentamidine, phenazopyridine, phthalylsulfatehiazole, pyrimethamine, salazosulfapyridine, sulfacarbamide, sulfacetamide, sulfachloropyridazine, sulfadiazine,

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sulfadicramide, sulfadimethoxine, sulfaethidol, sulfafurazol, sulfaguanidine, sulfaguanol, sulfamethizol, sulfamethoxazol and cotrimoxazol, sulfamethoxydiazine, sulfamethoxypyridazine, sulfamoxol, sulfanilamide, sulfaperine, sulfaphenazol, sulfatehiazol, sulfisomidine, tinidazol, trimethoprim, etc.;

- at least one substance from the class of coronary dilators, such as bamifylline, benziodarone, carbochromes, dilazep, dipyridamol, etafenone, fendiline, hexobendine, imolamine, lidoflazine, nifedipine, oxyfedrine, pentaerythrityltetranitrate, perhexiline, prenylamine, propatylnitrate, racefemine, trolnitrate, verapamil, visnadine, etc.;
- at least one cytostatic, for example, from the group of alkylating agents, antibiotics, platinum compounds, hormones and their inhibitors, interferones, etc.; very frequently used substances of this kind are:
aclerubicine, azathioprine, bleomycine, busulfane, calciumfolinate, carboplatinum, carmustine, chloroambucil, cis-platinum, cyclophosphamide, cyt-arabine, daunorubicine, epirubicine, fluorouracil, fosfestrol, hydroxycarbamide, ifosfamide, lomustine, melphalane, mercaptopurine, methotrexate, mitomycine C, mitopodozide, mitramicyne, nimustine, pipobromane, prednimustine, procarbazine, testolactone, theosulfane, thiotepa, tioguanine, triaziquone, trofosfamide, vincristine, vindesine, vinblastine, zorubicine, etc.;
- an intestinal antiseptic, such as broxyquinoline, clioquinol, diodohydroxyquinoline, halquinol, etc.;
- at least one diuretic, such as acetazolamide, aminophylline, bendroflumethiazide, bumetanide, butizide,

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chloroazanile, chloromerodrine, chlorothiazide, chloro-
talidone, clopamide, clorexolone, cyclopenthiazide,
cyclothiazide, etacrynic acid, furosemide, hydrochloro-
thiazide, hydroflumethiazide, mefruside, methazolamide,
paraflutizide, polythiazide, quinethazone, spirono-
lactone, triamterene, trichloromethiazide, xipamide,
etc.;

- at least one ganglion blocker, such as gallamintri-
ethiodide, hexamethonium-chloride, mecamylamine, etc.;
- at least one substance for the therapy of arthritis,
preferably analgetics or for example allopurinol,
benzbromarone, colchicine, benziodarone, probenecide,
sulfinpyrazone, tenoxicam, etc.; in very many cases
allopurinol;
- at least one glucocorticoid, such as beclomethason,
betamethason, clocortolone, cloprednol, cortison, dexamethason (e.g. as a dexamethasonephosphate), fludrocortison, fludroxycortide, flumetason, fluocinolon-acetonide, fluocinonide, fluocortolon (e.g. as a fluocortoloncapronate or fluocortolontrimethylacetate), fluorometholon, fluprednidenacetate, hydrocortison (also as a hydrocortison-21-acetate, hydrocortison-21-phosphate, etc.), paramethason, prednisolon (e.g. in the form of methylprednisolon, prednisolon-21-phosphate, prednisolon-21-sulfobenzoate, etc.), prednison, prednylidene, pregnenolon, triamcinolon, triamcinolonacetonide, etc.;
- at least one agent with a putative anti-flew action, such as moroxydine;
- at least one haemostatic, such as adrenalon, ascorbic

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acid, butanol, carbazochrome, etamsylate, protamine, samatostatine etc.; thyroidal hormones and vitamins can be employed for this purpose as well;

- at least one hypnotic, from the class of barbiturates, benzodiazepines, bromo-compounds, ureids, etc., for example; quite commonly applied for this purpose are, e.g. acecarbromal, alimemazintartrate allobarbital, amobarbital, aprobarbital, barbital, bromo-isoval, brotizolam, carbromal, chloroalhydrate, chloroalodol, chlorobutanol, clomethiazol, cyclobarbital, diazepam, diphenhydramine, doxylamine, estazolam, ethchlorvynol, ethinamate, etomidate, flurazepam, glutethimide, heptabarb, hexobarbital, lormetazepam, malperol, meclozine, medozine, methaqualon, methyprylon, midazolam, nitrazepam, oxazepam, pentobarbital, phenobarbital, promethazine, propallylonal, pyrithyldion, secbuta-barbital, secobarbital, scopolamine, temazepam, triazolam, vinylbital, etc.; various extracts from balm-mint, valerian, and passiflora are also used;
- at least one immunoglobuline, from the IgA, IgE, IgD, IgG, IgM classes or an immunoglobuline fragment, such as a Fab- or Fab2-fragment, or the corresponding variable or hypervariable region, if required in combination with other agents and/or chemically, biochemically or genetically manipulated;

An immunoglobuline can be of the IgA, IgD and IgE, IgG (e.g. Ig G1, Ig G2, Ig G3, Ig G4) or IgM type. In the context of this application, any chemical or biochemical derivative of any immunoglobuline (Ig) is considered useful, for example, an Ig G-gamma chain, an Ig G-F(ab')₂ fragment, an Ig G-F(ab) fragment, an Ig G-Fc fragment, an Ig-kappa chain, a light chain of Ig-s (e.g. a kappa and

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lambda chain), but also even smaller immunoglobuline fragments, such as the variable or hypervariable regions, or artificial modifications of any of these substances.

- at least one substance with an immunostimulating activity, with an immunosuppressive potency, with a capability to give rise to the production of immunoglobulines or other immunologically active substances (endotoxines, cytokines, lymphokines, prostaglandines, leucotrienes, other immuno modulators or biological messengers), including vaccines. Antibodies against any of these substances can also be used; preferred are immunotransfersomes with or without endotoxines, cytokines, prostaglandines, leucotrienes, with other immunomodulators, immunologically active cellular or molecular fragments, as well as corresponding antagonists, derivatives or precursors; particularly preferred compounds are lipid A and other glycolipids, muraminic acid derivatives, trehalose derivatives, phythaemaglutinines, lectins, polyinosine, polycytidylic acid (poli I:C), dimepranol-4-acetamidobenzoate, erythropoietin, 'granulocyte-macrophage colony stimulating factor' (GM-CSF), interleukine I and II, III and VI, interferon alpha, beta and/or gamma, leucotriene A, B, C, D, E and F, propandiamine, prostaglandine A, B, C, D, E, F, and I (prostacycline), tumor necrosis factor-alpha (TNF-alpha), thromboxan B, as well as immunoglobulines of types IgA, IgE, IgD, IgG, IgM; furthermore, suitable tissue and plant extracts, their chemical, biochemical or biological derivatives or replacements, their parts, such as characteristic peptide chains, etc.; as immunosuppressives, ganciclovir, azathiiprin, cyclosporin, FK 506 etc. are frequently used;

- at least one contraceptive agent, such as medroxyprogesteronacetate, lynesterol, lvonorgestrel, norethisteron, etc.;
- at least one circulation analeptic, such as cafedrin, etamivan, etilefrin, norfenefrin, pholedrin, theodrenalin, etc.;
- at least one drug for the therapy of liver diseases, such as orazamide, silymarin, or tiopromin;
- at least one substance with a light-protective function, such as mexenone;
- at least one antimalaria agent, such as amodiaquin, hydroxychloroquin or mepacrin;
- at least one substance for migraine or schizophrenia treatment, such as certain analeptics, beta-blockers, clonidin, dimetotiazine, ergotamine, lisurid (hydrogen maleate), methysergide, pizotifen, propranolol, proxibarbal, etc. Even more suitable are the serotonin antagonists or the blockers of serotonin receptors, such as 5-HT₁, 5-HT₂ or 5-HT₃; well suited for use according to this invention are also the receptor blockers AH21467 (Glaxo), AH25086 (Glaxo), GR43175 (Glaxo), GR38032 (Glaxo, = ondansetron), 5-hydroxytryptamine, ketanserin, methiothepin, alpha-methyl-5HT, 2-methyl-5HT, etc.;
- at least one mineral corticoid, such as aldosterone, fludrocortison, desoxycortonacetate, corresponding derivatives, etc.;
- at least one morphine antagonist (such as amiphenazol, lealvallorphan, nalorphine) or some substance with

morphine-like properties such as casomorphine, cyclo(leu-gly), dermorphine, met-enkephaline, methorphanide (tyr-gly-gly-phe-met-arg-arg-val), morphiceptine, morphine modulating neuropeptide (ala-gly-glu-gly-leu-ser-ser-pro-phe-trp-ser-leu-ala-ala-pro-gln-arg-phe-NH₂) etc.;

- at least one muscle relaxant, which frequently belongs to the groups of competitively or depolarising curare-agents, myotonolytics or analgetics; suitable substances with the desired effect are, among other materials, acetylsalicylic acid, alcuronium-chloride, azapropazon, atracuriumbesilate, baclofen, carisoprodol, quinine derivatives, chloromezalon, chlorophenesincarbamate, chlorozoxazon, dantrolen, decamethoniumbromide, dimethyltubocurariniumchloride, fenylamidol, gallamintriethiodide, guaiphensine, hexafluorenum-bromide, hexacarbacholinbromide, memantin, mephensesin, meprobamate, metamisol, metaxalon, methocarbamol, orphenadrin, paracetamol, phenazon, phenprobamate, suxamethoniumchloride, tetrazepam, tizanidin, tubocurarinchloride, tybamate, etc.;
- at least one narcotic, such as alfentanil, codeine, droperidol, etomidate, fentanil, flunitrazepam, hydroxybutiric acid, ketamine, methohexital, midazolam, thebacon, thiamylal, thiopental, etc., as well as corresponding derivatives;
- at least one substance with a neurotherapeutic activity, such as anaesthetics and vitamins, atropine-derivatives, benfotiamine, choline-derivatives, caffeine, cyanocobalamine, alpha-liponic acid, mepivacaine, phenobarbital, scopolamine, thiaminchloride hydrochloride, etc., and, most notably, procaine;

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- at least one neuroleptic, e.g. butyrophenon-derivatives, phenotiazin-derivatives, tricyclic neuroleptics, as well as acetophenazine, benperidol, butaperazine, carfenazine, chloropromazine, chlorprothixen, clopenthixol, clozapine, dixyrazine, droperidol, fluanison, flupentixol, fluphenazine, fluspirilen, haloperidol, homofenazine, levomepromazine, melperon, moperon, oxipertin, pecazine, penfluridol, periciazine, perphenazine, pimozide, pipamperon, piperacetazine, profenamine, promazine, prothipendyl, sulforidazine, thiopropazate, thioproperazine, thioridazine, tiotixen, trifluoperazine, trifluoperidol, triflupromazine, etc.; in particular, haloperidol and sulperide are often used for this purpose;
- at least one neurotransmitter or one of its antagonists; preferably, acetylcholine, adrenaline, curare (and, e.g. its antagonist edrophonium-chloride), dopamine, ephedrine, noradrenaline, serotonin, strychnine, vasotonine, tubocurarine, yohimbine, etc. are used;
- at least one opthalmic, in many cases from the groups of anaesthetics, antibiotics, corticoids, eye-tonics, chemotherapeutics, glaucoma agents, virustatics, antiallergics, vasodilatators, or vitamins;
- at least one parasympathicomimetic (e.g. bethanechol-chloride, carbachol, demecarium-bromide, distigmin-bromide, pyridostigmin-bromide, scopolamine) or at least one parasympathicolytic (such as benzatropine, methscopolamine-bromide, pilocarpine or tropicamide);
- at least one agent for the therapy of psoriasis and/or neurodermitis; particularly well suited for this purpose are carrier substances with a hypoallergic action or the corresponding edge active compounds, with n-3 (omega 3),

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less frequently with n-6 (omega 6), mainly with multiple, often 3-6, double bonds and/or hydroxy, more seldom methyl-, or oxo-side groups; these can also appear as side chains on further agent molecules; side groups on the 15th carbon atom are particularly efficient; as additives, amongst other substances, antimycotics, cytostatics, immunosuppressants or antibiotics can be used;

- at least one agent for the dilatation of the iris (mydriatic), such as atropine, atropinmethonitrate, cyclopentolate, pholedrine, scopolamine or tropicamide;
- at least one substance with a psychostimulating action; well suited for this purpose are, for example, amphetaminil, fencamfamine, fenetylline, meclofenoxate, methamphetamine, methylphenidate, pemoline, phendimetrazine, phenmetrazine, prolintane or viloxazine;
- at least one rhinologic, such as buphenine, cafaminol, carbinoxamide, chlorophenamim, chlorotenoxazine, clemastine, dextromethorpane, etilefrine, naphazoline, norephedrine, oxymetazoline, phenylaprhine, piprinydrinate, pseudoephedrine, salicylamide, tramazoline, triprolidine, xylometazoline, etc.; from biological sources especially the radix gentiane extract;
- at least one somnifacient (such as sleep-inducing peptide (trp-ala-gly-gly-asp-ala-ser-gly-glu)), or a corresponding antagonist (such as bemegride);
- at least one sedative or tranquilizer, as the former, for example, acecarbromal, alimemazine, allobarbital, aprobarbital, benzoctamine, benzodiazepine-derivatives,

bromo-isoval, carbromal, chloropromazine, clomethiazol, diphenyl-methane-derivatives, estazolam, fenetylline, homofenazine, mebutamate, mesoridazine, methylpentynol, methylphenobarbital, molindone, oxomemazine, perazine, phenobarbital, promethazine, prothipendyl, scopolamine, secbutabarbital, trimetozine, etc.; as a tranquilizer, for example, azacyclonol, benactyzin, benzoctamine, benzquinamide, bromo-azepam, chlorodiazepoxide, chlorophenesincarbonate, cloxazolam, diazepam, dipotassium-chloroazepate, doxepine, estazolam, hydroxyzine, lorazepam, medazepam, meprobamate, molindone, oxazepam, phenaglycodol, phenprobamate, prazepam, prochloroperazine, rescinnamine, reserpine or tybamate; drugs, such as distraneurine, hydantoine-derivatives, malonyl uric acid-derivatives (barbiturates), oxazolidine-derivatives, scopolamine, valepotriate, succinimide-derivatives, or hypnotics (e.g. diureides (such as barbiturates)), methaqualon, meprobromate, monoureides (such as carbromal), nitrazepam, or piperidin-dione, can be used for this purpose; amongst other substances, certain thymoleptics, such as librium or tofranil, can be used as antidepressants;

- at least one substance from the class of spasmolytics, e.g. adiphenine, alverine, ambicetamide, aminopromazine, atropine, atropine methonitrate, azintamide, bencyclane, benzarone, bevonium-methylsulfate, bietamiverine, butetamate, butylscopolammoniumbromide, camylofine, carzenide, chlorodiazepoxide, cionium-bromide, cyclandelate, cyclopentolate, dicycloverine, diisopromine, dimoxyline, diphemanil-methylsulfate, ethaverine, ethenzamide, fencarbamide, fenpipramide, fenpivennum-bromide, gefarnate, glycopyrroniumbromide, hexahydroadiphenin, hexocycliummethylsulfate, hymecromon,

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isometheptene, isopropamidiodide, levomethadone, mebeverine, metamizon, methscopolamine-bromide, metixen, octatropine-methylbromide, oxazepam, oxybutin, oxyphenonium-bromide, papaverine, paracetamol, pentapiperide, penthienate-methobromide, pethidine, pipenzolate-bromide, piperidolate, pipoxolane, propanthelin-bromide, propylphenazon, propyromazine-bromide, racefemine, scopolamine, sulpiride, tiemonium-iodide, tridihexethyliodide, tropenzilinbromide, tropinbenzilate, trospiumchloride, valetamatbromide, etc.; furthermore, belladonna alkaloids, papaverine and its derivatives, etc.;

- at least one sympathicolytic, e.g. azapetine or phentolamine;
- at least one sympathicomimetic, e.g. bamethane, buphenine, cyclopentamine, dopamine, L-(-)-ephedrine, epinephrine, etilefrine, heptaminol, isoetarine, metaraminol, methamphetamine, methoxamine, norfenefrine, phenylpropanolamine, pholedrine, propylhexedrine, protokylol or synephrine;
- at least one tuberculostatic, such as an antibiotic, p-aminosalicylic acid, capreomycine, cycloserine, dapson, ethambutol, glyconiazide, iproniazide, isoniazide, nicotinamide, protionamide, pyrarinamide, pyrodoxine, terizidone, etc., and, particularly preferred thereof, ethambitol and isoniazide;
- at least one urologic, e.g. a bladder tension modifying agent (such as cholinecitrate, distigminebromide, yohimbine), a corresponding antiinfection agents (antibiotics, chemotherapeutics, or nitrofurantoid-, chinolone-, or sulfonamide-derivative); furthermore,

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adipinic acid, methionine, methenamine-derivatives, etc.;

- at least one substance with a vasoconstricting action; often, adrenalone, epinephrine, felypressine, methoxamine, naphazoline, oxymetazoline, tetrazyoline, tramazoline or xylometazoline are used for this purpose;
- at least one substance which is a vasodilatator, such as e.g. azapetine, banethane, bencyclane, benfurodil-hemisuccinate, buphenine, butalamine, cinnarizine, diprophylline, hexyltheobromine, ifenprodil, isoxsuprine, moxisylyte, naftidrofuryl, nicotinylalcohol, papaverine, phenoxybenzamine, piribedil, primaperone, tolazoline, trimetazidine, vincamine or xantinol-nicotinate;
- at least one veins agent, e.g. aescine, benzarone, calcium-dobesilate, dihydroergotaminemesilate, diosmine, hyhydroxyethylrutoside, pignogenol, rutoside-aesinate, tribenoside, troxerutine, etc.;
- at least one virustatic, e.g. one immunostimulating agent, and/or an additional drug, such as as moroxydine or tromantadine, which may stimulate action of the immunostimulator;
- one agent for the treatment of wounds; for example, dexpanthenol, growth stimulating factors, enzymes or hormones, especially in combination with carriers which contain essential substances; povidon-iodide, fatty acids which are not straight, cetylpyridiniumchloride, chinoline-derivatives of known antibiotics and analgetics are useful;
- at least one substance with a toxic action or a toxin; common toxins from plant or microbial sources in

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particular 15-acetoxyscirpenol, 3-acetyldeoxynivalenol, 3-alpha-acetyldiacetoxyscirpenol, acetyl T-2 toxin, aflatoxicol I, aflatoxicol II, aflatoxin B1, aflatoxin B2, aflatoxin B2-alpha, aflatoxin G1, aflatoxin G2, aflatoxin G2-alpha, aflatoxin M1, aflatoxin M2, aflatoxin P1, aflatoxin Q1, alternariol-monomethyl ether, aurovertin B, botulinum toxin D, cholera toxin, citreoviridin, citrinin, cyclopiazonic acid, cytochalasin A, cytochalasin B, cytochalasin C, cyrochalasin D, cytochalasin, cytochalasin H, cytochalasin J, deoxynivalenol, diacetoxyscirpenol, 4,15-diacetylverrucarol, dihydrocytochalasin B, enterotoxin STA, fusarenon X, iso T-2 toxin, O- methylsterigmatocystin, moniliformin, monoacetoxyscirpenol, neosolaniol, ochratoxin A, patulin, penicilinic acid, pertussis toxin, picrotoxin, PR-toxin, prymnesin, radicinin, roridin A, rubratoxin B, scirpentriol, secalonic acid D, staphylococcal enterotoxin B, sterigmatocystin, streptolysin O, streptolysin S, tentoxin, tetrahydrodeoxyaflatoxin B1, toxin A, toxin II, HT-2 toxin, T-2-tetraol, T-2 toxin, trichothecin, trichothecolon, T-2 triol, verrucaric acid, verrucarol, vomitoxin, zearalenol and zearalenon.

- at least one substance which affects growth in humans or animals, such as basic fibroblast growth factor (BFGF), endothelial cell growth factor (ECGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin, insulin-like growth factor I (LGF I), insulin-like growth factor II (LGF II), nerves-growth factor-beta (NGF-beta), nerves growth-factor 2,5s (NGF 2,5s), nerves growth-factor 7s (NGF 7s), platelet-derived growth factor (PDGF), etc.;
- a carrier and/or agent which creates a protective layer on and/or in a barrier, such as skin, against poison,

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light UV-, gamma- or other radiation; against detrimental biological agents such as viruses, bacteria, toxins, etc.; carrier components and/or agents can hamper the detrimental action by chemical, biochemical, or biological means or else may prevent or diminish the penetration of such adversary agents;

- at least one fungicide, herbicide, pesticide, or insecticide;
- at least one plant hormone, e.g. abscisic acid, abscisic acid-methylester, 3-acetyl-4-thiazolidine-carboxyl acid, 1-allyl-1-(3,7-dimethyloctyl)-piperidinium bromide, 6-benzylaminopurine, 6-benzylaminopurine 9-(beta-glucoside), butanedio acid mono(2,2-dimethyl hydrazide), chlorocholine chloride, 2-chloroethyl-tris-(2'-methoxyethoxy)silane, 2-(o-chlorophenoxy)-2-methylpropionic acid, 2-(p-chlorophenoxy)-2-methylpropionic acid, 2-(o-chlorophenoxy)propionic acid, 2-(m-chlorophenoxy)propionic acid, clofibrinic acid, colchicine, o-coumarinic acid, p-coumarinic acid, cycloheximide, alpha,beta-dichloroisobutiric acid, 2-(2,4-dichlorophenoxy)propanoic acid, 2,3-dihydro-5,6-diphenyl 1,4-oxathiine, dihydrozeatine, 6-(gamma,gamma-dimethylallylamino)purino riboside, 3-(2-[3,5-dimethyl-2-oxocyclohexyl-2-hydroxyethyl])-glutarimide, trans-2-dodecenedioic acid, ethyl-8-chloro-1-indazol-3-yl-acetate, N6-furfuryladenine, 6-furfurylamino-purineriboside, gibberellic acid methylester, gibberellin A3-acetate, gibberellin A1 methylester, gibberellin A4 methylester, gibberellin A5 methylester, gibberellin A7 methylester, gibberellin A9 methylester, gibberellin A3 methylester 3,13-diacetate gibberinic acid, allo-gibberinic acid, gibberinic acid methylester, glyoxim, 22(s),23(s)-homobrassinolide, 9-hydroxyfluorene 9-

carboxylate, indol-3-acetic acid, indol-3-acetic acid ethylester, indol-3-propanoic acid, N6-(2-isopentenyl)adenine, N6-(2-isopentenyl)adenosine, 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylat methylchloride, kinetinglucoside, kinetinriboside, melissylalcohol, 1-methyladenine, methyl 2-chloro-9-hydroxy-fluorene-9-carboxylate, methyl 3,6-dichloro-o-anisate, 6-methylmercaptapurine, 1-naphthylacetamide, nonanoic acid methylester, 6-piperidino-1-purine, n-triacontanol, (-)-xanthoxine, zeatine glucosides, etc.;

- at least one pheromone or one pheromone-like substance, such as (-)-bornyl acetate, trans-5-decenol, cis-5-decenyl acetate, trans-5-decenyl acetate, 2,6-dichlorophenol, 1,7-dioxaspiro[5.5]undecane, trans-8,trans-10-dodecadienol ([E,E]-8,10-DDDOL), trans-7, cis-9-dodecadienyl acetate ([E,Z]-7,9-DDDA), trans-8, trans-10-dodecadienyl acetate ([E,E]-8,10-DDDA), cis-7-dodecen-1-ol (Z-7-DDOL), trans-10-dodecenol, cis-7-dodecenyl acetate (Z-7-DDA), cis-8-dodecenyl acetate, trans-8-dodecenyl acetate, 11-dodecenyl acetate, cis-7,8-epoxy-2-methyl-octadecane, cis-9-heneicosene, cis-7,cis-11-hexadecadienylacetate ([Z,Z]-7,11-HDDA), cis-7,trans-11- hexadecadienyl acetate ([Z,E]-7,11-HDDA), cis-9-hexadecenal (Z-9-HDAL), cis-11-hexadecenal (Z-11-HDAL), cis-11-hexadecenol (Z-11-HDOL), cis-11-hexadecenyl acetate (Z-11-HDA), trans-2-hexenyl acetate, cis-7-tetradecenal (Z-7-TDAL), cis-9-tetradecenol (Myristoleyl alcohol; Z-9-TDOL), cis-7-tetradecenol (Z-7-TDOL), cis-11-tetradecenol, cis-7-tetradecenyl acetate (Z-7-TDA), cis-9-tetradecenyl acetate (Myristoleyl acetate; Z-9-TDA), cis-11-tetradecenyl acetate (Z-11-TDA), trans-11-tetradecenyl acetate (E-11-TDA), cis-9-tetradecenyl formate

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(Myristoleyl formate; Z-9-TDF), isoamyl acetate (acetic acid 3-methylbutyl ester), 2-methyl-3-buten-2-ol, 3-methyl-2-cyclohexen-1-ol, cis-14-methyl-8-hexadecenal, cis-2-methyl-7-octadecene, 4-methylpyrrole-2-carboxylic acid methyl ester (Methyl 4-methylpyrrole 2-carboxylate) cis-13-octadecenal 13-octadecyn-1-ol, 2-(phenyl)ethyl propionate (phenylethanol propanoate), propyl cyclohexylacetate, cis-9,trans-11-tetradecadienol ([Z,E]-9,11-TDDOL), cis-9,trans-11-tetradecadienyl acetate ([Z,E]-9,11-TDDA), cis-9,trans-12-tetradecadienyl acetate ([Z,E]-9,12-TDDA), trichloroacetic acid esters, cis-9-tricosene, undecanal, etc.;

- at least one pigment or one colouring substance;
- at least one carbohydrate;

A carbohydrate, normally, has a basic formula $C_x(H_2O)_y$, e.g. in sugar, starch, cellulose, and, moreover, can be derivatised in many different ways.

A monomeric carbohydrate residue is, for example, a natural monosaccharide residue, which in many cases is an adduct of a pentose or a hexose in aldose or ketose form which, in principle, can adopt L- or D-configurations. Owing to the space constraints and due to their greater biological relevance, only the latter will be referred to in the following.

An aldose with five carbon atoms (aldo-pentose, or simply pentose) is for example D-arabinose, D-lyxose, D-ribose or D-xylose.

A ketose with five carbon atoms (keto-pentose) is e.g. D-ribulose or D-xylulose.

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A hexose, very frequently, exists in a cyclic form, as a pyranose (aldose), for example; alpha- or beta-D-glucopyranose are two typical examples for this. Another type of hexose is furanose, e.g. in an alpha- or beta-D-fructose. The pyranosyl residue is particularly preferably conjugated to a hydroxy group, the latter then being located in 1- or 6-positions; the furanosyl residue is preferably conjugated to the corresponding groups in positions 1- or 5-.

A carbohydrate residue, moreover, can be a natural disaccharide residue, e.g. a disaccharide residue consisting of two hexoses. Such a disaccharide residue arises, for example, through condensation of two aldoses, e.g. D-galactose or D-glucose, or one aldose, e.g. D-glucose and one ketose, e.g. fructose; disaccharides formed from two aldoses, such as lactose or maltose, are preferably conjugated to the phosphatidyl group through the hydroxy group, which is located in position 6- of the corresponding pyranosyl residue. A disaccharide formed from an aldose and a ketose, such as saccharose, is preferably conjugated through a hydroxyl-group in position 6- of the pyranosyl residue or in position 1- of the furanosyl residue.

A carbohydrate residue, moreover, is any derivatised mono-, di- or oligosaccharide residue, in which, for example, an aldehyde group and/or one or two terminal

hydroxy groups are oxidized to carboxy groups, e.g. in a D-glucar-, D-glucon- or D-glucuronic acid residue, all such residues being normally in the form of cyclic lactone residues. The aldehyde- or keto-groups in a derivatised mono- or disaccharide residue, moreover, can be reduced to hydroxy groups, e.g. in inositol, sorbitol or D-mannitol. Furthermore, individual hydroxy groups can be replaced by hydrogen atoms, e.g. in desoxysugars, such as 2-desoxy-D-ribose, L-fucose or L-rhamnose, or through amino groups, e.g. in aminosugars, such as D-galactosamine or D-glucosamine.

A carbohydrate can result from a cleaving action, starting with one of the mentioned mono- or disaccharides, by a strong oxidation agent, such as periodic acid. Amongst the biologically most important or most active carbohydrates are e.g. 2-acetamido-N-(epsilon-amino-caproyl)-2-deoxy-beta-glucopyranosylamine, 2-acetamido-1-amino-1,2-dideoxy-beta-glucopyranose, 2-acetamido-1-beta-(aspartamido)-1,2-dideoxyglucose, 2-acetamido-4,6-o-benzyliden-2-deoxy-beta-glucopyranose, 2-acetamido-2-deoxyallose, 3-acetamido-3-deoxyallose, 2-acetamido-2-deoxy-3-o-(beta-galactopyranosyl)-galactopyranose, 2-acetamido-2-deoxy-4-o-([4-o-beta-galactopyranosyl-beta-galactopyranosyl]-beta-galactopyranosyl)-glucopyranose, 2-acetamido-2-deoxy-3-o-(beta-galactopyranosyl)-alpha-glucopyranose, 6-o-(2-acetamido-2-deoxy-4-o-[beta-galactopyranosyl]-beta-glucopyranosyl)-galactopyranose, 4-o-acetamido-2-deoxy-6-o-(beta-galacto-4-o-(6-o-[2-acetamido-2-deoxy-beta-glucopyranosyl]-beta-galactopyranosyl) glucopyranose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, 3-acetamido-3-deoxyglucose pyranose, 6-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-galactopyranose, 2-acetamido-2-deoxy-1-thio-beta-glucopyranose 3,4,6-

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triacetate, acetopyruvic acid, N-acetylchondrosamine, N-acetylgalactosamine, N-acetylglucosamine, N-acetyl-alpha-glucosamine 1-phosphate, N-acetylglucosamine 6-phosphate, N-acetylglucosamine 3-sulfate, N-acetylglucosamine 6-sulfate, N-acetylheparine, N-acetylactosamine, N-acetyl-beta-mannosamine, N-acetylneuraminic acid, N-acetylneuramine-lactose, 1-o-acetyl-2,3,5-tri-o-benzoyl-beta-ribofuranose, trans-aconic acid, adenine-9-beta-arabinofuranoside, adenosine 5'-diphosphoglucose, adenosine 5'-diphosphomannose, adonite, adonitol, adonose, agar, algin, alginic acid, beta-allose, alpha glycerophosphate, alpha ketoglutaric acid, altrose, (-)-altrose, p-aminobenzyl-1-thio-2-acetamido-2-deoxy-beta-glucopyranoside, N-epsilon-aminocaproyl-beta-fucopyranosylamine, N-epsilon-aminocaproyl-alpha-galactopyranosylamine, 2-amino-2-deoxygalactopyranose, 6-amino-6-deoxyglucopyranose, 1-amino-1-deoxy-beta-glucose, 6-aminohexyl-N-acetyl-beta-thioglucoaminide, 6-aminohexyl-1-thio-beta-galactopyranoside, 5-aminoimidazole-4-carboxamidoxime-1-beta-ribofuranosyl 3':5'-cyclo-monophosphate, delta-aminolevulinic acid, p-aminophenyl-2-acetamido-2-deoxy-beta-glucopyranoside, p-aminophenyl-2-acetamido-2-deoxy-1-thio-beta-glucopyranoside, p-aminophenyl-alpha-fucopyranoside, p-aminophenyl-alpha-galactopyranoside, p-aminophenyl-beta-galactopyranoside, p-aminophenyl-alpha-glucopyranoside, p-aminophenyl-beta-glucopyranoside, c-aminophenyl-beta-glucuronide, p-aminophenyl-1-thio-beta-glucuronide, p-aminophenyl-beta-lactopyranoside, p-aminophenyl-alpha-mannopyranoside, p-aminophenyl-beta-thiofucopyranoside, p-aminophenyl-1-thio-beta-galactopyranoside, p-aminophenyl-1-thio-beta-glucopyranoside, p-aminophenyl-1-thio-beta-xylopyranoside, p-aminophenyl-beta-xylopyranoside, 5-amino-1-(beta-ribofuranosyl)imidazole 4-carboxamide, amygdaline, n-amyl beta-glucopyranoside, amylopectine, amylose, apigenine 7-

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o-hesperidoside, arabinitol, arabinocytidine, 9-beta-arabinofuranosyladenine, 1-beta-arabinofuranosylcytosin, arabinose, arabinose 5-phosphate, arabinosylcytosine, arabite, arabitol, arbutine, atp-ribose, atractyloside, aurothioglucose, n-butyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, calcium gluconate, calcium heptagluconate, carboxyatractyloside, carboxymethylamylose, carboxymethylcellulose, carboxyethylthioethyl-2-acetamido-2-deoxy-4-o-beta-galactopyransol-beta-glucopyranoside, carboxyethylthioethyl 4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyranosyl)-beta-glucopyranoside, 4-o-(4-o-[6-o-beta-D-galactopyranosyl-beta-D-galactopyranosyl]-D-glucopyranose, carrageenan, D(+)cellobiose, D(+)cellopentaose, D(+)cellotetraose, D(+)cellotriose, cellulose, cellulose caprate, cellulose carbonate, chitin, chitobiose, chitosan, chitotriose, alpha-chloroalose, beta-chloroalose, 6-chloro-6-deoxy-alpha-glucopyranose, chondroitin sulfate, chondrosamine, chondrosine, chrysophanic acid, colominic acid, convallatoxin, alpha-cyclodextrine, beta-cyclodextrine, cytidine 5'-diphosphoglucose, cytosine 1-beta-arabinofuranoside, daunosamine, n-decyl-beta-glucopyranoside, 5-deoxyarabinose, 2-deoxy-2-fluoroglucose, 3-deoxy-3-fluoroglucose, 4-deoxy-4-fluoroglucose, 6-deoxygalactopyranose, 2-deoxygalactose, 1-deoxyglucohex-1-eno-pyranose tetrabenzoat, 2-deoxyglucose, 6-deoxyglucose, 2-deoxyglucose 6-phosphate, 1-deoxymannojerimycin, 6-deoxymannose, 1-deoxy-1-morpholinofructose, 1-deoxy-1-nitroalutol, 1-deoxy-1-nitroaltitol, 1-deoxy-1-nitrogallactitol, 1-deoxy-1-nitromannitol, 1-deoxy-1-nitrosorbitol, 1-deoxy-1-nitrotalitol, deoxynojirimycine, 3-deoxy-erythro-pentose, 2-deoxy-6-phosphogluconic acid, 2-deoxyribose, 3-deoxyribose, 2-deoxy-alpha-ribose 1-

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phosphate, 2-deoxyribose 5-phosphate, 5-deoxyxylofuranose, dextran, dextransulfate, dextrine, dextrose, diacetonefructose, diacetone mannitol, 3,4-di-o-acetyl-6-deoxyglucal, di-o-acetyl rhamnal, 2,3-diamino-2,3-dideoxy-alpha-glucose, 6,9-diamino-2-ethoxyacridine lactate, 1,3:4,6-di-o-benzylidene mannitol, 6,6'-dideoxy-6,6'-difluorotrehalose, digalactosyl diglyceride, digalacturonic acid, (+)digitoxose, 6,7-dihydrocoumarin-9-glucoside, dihydroxyacetone, dihydroxyacetone phosphate, dihydroxyfumaric acid, dihydroxymalic acid, dihydroxytartaric acid, dihydrozeatinriboside, 2,3-diphosphoglycerolic acid, dithioerythritol, dithiothreitol, n-dodecyl beta-glucopyranoside, n-dodecyl beta-maltoside, dulcitol, elemi-gum, endotoxin, epifucose, erythritol, erythro-pentulose, erythrose, erythrose 4-phosphate, erythrulose, esculin, 17-beta-estradiol-3-glucuronide 17-sulfate, estriole glucuronide, estron beta-glucuronide, ethodin, ethyl 4-o-beta-D-galactopyranosyl)-beta-D-glucopyranoside, ethyl 2-acetamido-4-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-6-o-(alpha-fucopyranosyl)-2-deoxy-beta-glucopyranoside, ethyl 2-acetamido-2-deoxy-4-o-(4-o-alpha-galactopyranosyl-beta-galactopyranosyl)-beta-glucopyranoside, ethyl cellulose ethylene glycol chitin, ethyl 4-o-(4-o-alpha-galactopyranosyl-beta-galactopyranosyl)-beta-glucopyranoside, ethyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, ethyl pyruvate, ethyl beta-thiogluconide, etiocholane-3alpha-ol-17-on glucuronide, ficoll, 6-fluoro-6-deoxyglucose, franguloside, fraxin, fructosazine, beta-(-)fructose, fructose-1,6-diphosphate, fructose-2,6-diphosphate, fructose-1-phosphate, fructose-6-phosphate, fucoidan, fucose, alpha-(-)-fucose-1-phosphate, fucosylamine, 2'-fucosyllactose, 3-fucosyllactose, fumaric acid, galactal,

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galactitol, galactopyranosylamine, 3-o-beta-galactopyranosyl-arabinose, 4-o-beta-galactopyranosyl-fructofuranose, 4-o-(4-o-beta-galactopyranosyl beta-galactopyranosyl)-glucopyranose, 4-o-alpha-galactopyranosyl-galactopyranose, 6-o-beta-galactopyranosylgalactose, 4-o-(beta-galactopyranosyl)-alpha-mannopyranose, alpha-galactopyranosyl 1-phosphate, galactopyranosyl-beta-thio-galactopyranoside, (+)galactosamine, alpha-galactosamine 1-phosphate, alpha-galactose 1-phosphate, galactose 6-phosphate, galactose 6-sulfate, 6-(alpha-galactosido)glucose, galacturonic acid, beta-gentiobiose, glucan, glucitol, glucoheptonic acid, glucoheptose, glucoheptulose, gluconate 6-phosphate, gluconic acid, 1-o-alpha-glucopyranosyl-beta-fructofuranoside, 6-o-alpha-glucopyranosylfructose, 1-o-alpha-glucopyranosyl-alpha-glucopyranoside, 4-o-beta-glucopyranosylglucopyranose, 4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyranosyl) glucopyranose, (+)glucosamine, alpha-glucosamine 6-2,3-disulfate, alpha-glucosamine 1-phosphate, glucosamine 6-phosphate, glucosamine 2-sulfate, alpha-glucosamine 3-sulfate, glucosamine 6-sulfate, glucosaminic acid, glucose, alpha-glucose 1,6-diphosphate, glucose 1-phosphate, glucose 6-phosphate, glucose 6-sulfate, glucuronamide, glucuronic acid, alpha-glucuronic acid 1-phosphate, glyceraldehyde, glyceraldehyde 3-phosphate, glycerate 2,3-diphosphate, glycerate 3-phosphate, glyceralic acid, alpha-glycerophosphate, beta-glycerophosphate, glycogen, glycolaldehyde, glycol chitosan, n-glycolylneuraminic acid, glycyric acid, glyoxylic acid, guanosine, 5'-diphosphoglucose, gulose, gums (accroides, agar, arab, carrageenan, damar, elemi, ghatti, guaiac, guar, karaya, locust bonne, mast, pontianac, storax, tragacanth, xanthan), heparin and heparin-like substances

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(mesoglycan, sulodexide, etc.), heptakis (2,3,6-tri-o-methyl)-beta-cyclodextrin, heptanoyl-N-methylglucamide, n-heptyl beta-glucopyranoside, hesperidin, n-hexyl-beta-glucopyranoside, hyaluronic acid, 16-alpha-hydroxyestronglucuronide, 16-beta-hydroxyestron glucuronide, hydroxyethyl starch, hydroxypropylmethyl-cellulose, 8-hydroxyquinolin-beta-glucopyranoside, 8-hydroxyquinolin glucuronide, idose, (-)-idose, indole-3-lactic acid, indoxyl-beta-glucoside, epi-inositol, myo-inositol, myo-inositol bisphosphate, myo-inositol-1,2-cyl phosphate, scyllo-inositol, inositolhexaphosphate, inositolhexasulfate, myo-inositol 2-monophosphate, myo-inositol trisphosphate, (q)-epi-inosose-2, scyllo-inosose, inulin, isomaltose, isomaltotriose, isosorbide dinitrate, 11-ketoandrosterone beta-glucuronide, 2-ketogluconic acid, 5-ketogluconic acid, alpha-ketopropionic acid, lactal, lactic acid, lactitol, lactobionic acid, lacto-N-tetraose, lactose, alpha-lactose 1-phosphate, lactulose, laminaribiose, laminarine, levoglucosan, beta-levulose, lichenan, linamarine, lipopolysaccharides, lithiumlactate, lividomycine A, lyxose, lyxosylamine, maltitol, maltoheptaose, maltohexaose, maltooligosaccharide, maltopentaose, maltose, alpha-(+)maltose 1-phosphate, maltotetraose, maltotriose, malvidine-3,5-diglucoside, mandelonitril beta-glucoside, mandelonitril glucuronic acid, mannan, mannitol, mannitol 1-phosphate, alpha-mannoheptitol, mannoheptulose, 3-o-alpha-mannopyranosyl-mannopyranose, alpha-(+)mannopyranosyl-1-phosphate, mannosamine, mannosan, mannose, A-(+)mannose 1-phosphate, mannose 6-phosphate, (+)melezitose, A-(+)melibiose, mentholglucuronic acid, 2-(3'-methoxyphenyl)-N-acetylneuraminic acid, methyl 3-o-(2-acetamido-2-deoxy-beta-galactopyranosyl)-alpha-

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galactopyranoside, methyl 4-o-(3-o-[2-acetamido-2-deoxy-4-o-beta-galactopyranosyl beta-glucopyranosyl]-beta-galactopyranosyl)-beta-glucopyranoside, methyl 2-acetamido-2-deoxy-beta-glucopyranoside, methyl 3-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-beta-galactopyranoside, methyl 6-o-(2-acetamido)-2-deoxy-beta-glucopyranosyl)-alpha-mannopyranoside, methyl acosaminide, methyl alpha-altropyranoside, methyl 3-amino-3-deoxy-alpha-mannopyranoside, methyl beta-arabinopyranoside, methyl 4,6-o-benzylidene-2,3-di-o-toluenesulfonyl-alpha-galactopyranoside, methyl 4,6-o-benzylidene-2,3-di-o-p-toluenesulfonyl-alpha-glucopyranoside, methyl cellulose, methyl alpha-daunosaminide, methyl 6-deoxy-alpha-galactopyranoside, methyl 6-deoxy-beta-galactopyranoside, methyl 6-deoxy-alpha-glucopyranoside, methyl 6-deoxy-beta-glucopyranoside, methyl 3,6-di-o-(alpha-mannopyranosyl)-alpha-mannopyranoside, 1-o-methyl-alpha-galactopyranoside, 1-o-methyl-beta-galactopyranoside, methyl 3-o-alpha-galactopyranosyl-alpha-galactopyranoside, methyl-3-o-beta-galactopyranosyl-beta-galactopyranoside, 4-o-(2-o-methyl-beta-galactopyranosyl) glucopyranose, methyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, methyl-4-o-(beta-galactopyranosyl-alpha-mannopyranoside, 5-5-methylgalactopyranose, methylgalactoside, n-methylglucamine, 3-o-methyl-alpha-glucopyranose, 1-o-methyl-alpha-glucopyranoside, 1-o-methyl-beta-glucopyranoside, alpha-methyl glucoside, beta-methyl glucoside, methyl glycol chitosan, methyl-alpha-mannopyranoside, methyl-2-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl 3-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl-4-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl 6-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl alpha-rhamnopyranoside, methyl alpha-ribofuranoside, methyl beta-ribofuranoside,

methyl-beta-thiogalactoside, methyl 2,3,5-tri-O-benzoyl-alpha-arabinofuranoside, 4-methylumbelliferyl-2-acetamido-4,6-O-benzylidene-2-deoxy-beta-glucopyranoside, 4-methylumbelliferyl N-acetyl-beta-galactosaminide, 4-methylumbelliferyl N-acetyl-alpha-glucosaminide, 4-methylumbelliferyl-N-acetyl-beta-glucosaminide, 4-methylumbelliferyl-alpha-arabinofuranoside, 4-methylumbelliferyl-alpha-arabinopyranoside, 4-methylumbelliferyl-beta-cellobioside, 4-methylumbelliferyl-beta-D-glucopyranoside, 4-methylumbelliferyl-alpha-fucoside, 4-methylumbelliferyl-beta-fucoside, 4-methylumbelliferyl-alpha-galactopyranoside, 4-methylumbelliferyl-beta-galactopyranoside, 4-methylumbelliferyl-alpha-galactoside, 4-methylumbelliferyl-beta-glucopyranoside, 4-methylumbelliferyl-alpha-glucoside, 4-methylumbelliferyl-beta-glucoside, 4-methylumbelliferyl-beta-glucuronide, 4-methylumbelliferyl-beta-mannopyranoside, 4-methylumbelliferyl-beta-D-glucopyranoside, 4-methylumbelliferyl-2,3,5-tri-O-benzyl-alpha-arabinofuranoside, 4-methylumbelliferyl-beta-xyloside, methyl-beta-xylopyranoside, 2-O-methylxylose, alpha-methylxyloside, beta-methylxyloside, metrizamide, 2'-monophosphoadenosine 5'-diphosphoribose, 2'-monophosphoinosine 5'-diphosphoribose, mucine, muraminic acid, naringine, sodium lactate, sodium polypectate, sodium pyruvate, neoagarobiose, neoagarohexaitol, neoagarohexaose, neoagarotetraose, beta-neocarrabiose, neocarrabiose 4/1-sulfate, neocarrahexaose(2/4,4/1,4/3,4/5)-tetrasulfate, neocarratetraose(4/1,4/3)-disulfate, neocarratetraose(4/1)-sulfate, neohesperidin, dihydrochalcon, neohesperidose, neuraminic acid, neuraminic acid beta-methylglycoside, neuramine-lactose, nigeran, nigerantetrasaccharide, nigerose, n-nonyl glucoside, n-nonyl-beta-glucopyranoside, octadecylthioethyl 4-O-alpha-galactopyranosyl-beta-galactopyranoside,

octadecylthioethyl 4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyranosyl)-beta-glucopyranoside, octanoyl n-methylglucamide, n-octyl alpha-glucopyranoside, n-octyl-beta-glucopyranoside, oxidised starch, pachyman, palatinose, panose, pentaerythritol, pentaerythritol diformal, 1,2,3,4,5-pentahydroxy, capronic acid, pentosanpolysulfate, perseitol, phenolphthalein glucuronic acid, phenolphthalein mono-beta-glucosiduron phenyl 2-acetamido-2-deoxy-alpha-galactopyranoside, phenyl 2-acetamido-2-deoxy-alpha-glucopyranoside, alpha-phenyl-N-acetyl-glucosaminide, beta-phenyl N-acetyl-glucosaminide, phenylethyl beta-galactoside, phenyl beta-galactopyranoside, phenyl beta-galactoside, phenyl alpha-glucopyranoside, phenyl beta-glucopyranoside, phenyl alpha-glucoside, phenyl beta-glucoside, phenyl beta-glucuronide, beta-phenyllactic acid, phenyl alpha-mannopyranoside, beta-phenylpyruvic acid, phenyl beta-thiogalactopyranoside, phenyl beta-thiogalactoside, phospho(enol)pyruvate, (+)2-phosphoglyceric acid, (-)3-phosphoglyceric acid, phosphohydroxypyruvic acid, 5-phosphorylribose 1-pyrophosphate, phytic acid, poly-N-acetylglucosamine, polygalacturonic acid, polygalacturonic acid methyl ester, polypectate, sodium, polysaccharide, 5beta-pregnane-3alpha,20alpha-diol glucuronide, n-propyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, prunasine, psicose, pullulan, quinolyl-8beta-glucuronic acid, (+)raffinose, alpha-rhamnose, rhapontine, ribitol, ribonolacton, ribose, D-2-ribose, alpha-ribose 1-phosphate, ribose 2-phosphate, ribose 3-phosphate, ribose 5-phosphate, ribulose, ribulose-1,5-diphosphate, ribulose 6-phosphate, saccharic acid, saccharolactic acid, saccharose, salicin, sarcolactic acid, schardingers-alpha-dextrine, schardingers-beta-dextrine,

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sedoheptulosan, sedoheptulose 1,7-diphosphate, sialic acid, sialyllactose, sinigrine, sorbitol, sorbitol 6-phosphate, (+)-sorbitol, (-)-sorbitol, stachyose, starch, storax, styrax, sucrose, sucrose monocaprinate, tagatose, alpha-talose, (-)-talose, tartaric acid, testosterone-beta-glucuronide, 2,3,4,6-tetra-O-methyl-glucopyranose, thioglucoside, 1-thio-beta-galactopyranose, beta-thioglucose, 5-thioglucose, 5-thioglucose 6-phosphate, threitol, threose, (+)-threose, (-)-threose, thymidine 5'-diphosphoglucose, thymine 1-beta-arabinofuranoside, tragacanth, (+)-trehalose, trifluorothymine, deoxyriboside, 3,3',5-trihydroxy-4'-methoxy-stilbene-3-O-beta-glucoside, trimethylsilyl(+)-arabinose, trimethylsilyldulcitol, trimethylsilyl-beta(-)-fructose, trimethylsilyl(+)-galactose, trimethylsilyl-alpha(+)-glucose, trimethylsilyl(+)-mannitol, trimethylsilyl(+)-rhamnose, trimethylsilyl(-)-sorbitol, trimethylsilyl(+)-xylose, rac-1-O-tritylglycerol, (+)-turanose, n-undecyl beta-glucopyranoside, uracil beta-arabinofuranoside, uridine 5'-diphospho-N-acetylglucosamine, uridine 5'-diphosphogalactose, uridine 5'-diphosphoglucose, uridine 5'-diphosphoglucuronic acid, uridine 5'-diphosphomannose, uridine 5'-diphosphoxylose, vancomycin, xanthan gum, xylane, xylite, xylitol, xylobiose, alpha-xylopyranosyl 1-phosphate, xylose, alpha-xylose 1-phosphate, xylose 5-phosphate, xylotriose, xylulose, xylulose 5-phosphate, yacca, zeatine riboside, zinclactate, zymosan A, etc.

Denotations desoxyribonucleic-(DNA) and ribonucleic acid (RNA) have their common meaning; preferably such DNA or RNA forms, or their antagonists, are used which have a particularly strong biological action.

- at least one nucleotide, peptide, protein or a related compound;

Nucleotides, which can be effectively transported with the aid of transfersomes, encompass adenine, adenosine, adenosine-3',5'-cyclic monophosphate, N6,02'-dibutyryl, adenosine-3',5'-cyclic monophosphate, N6,02'-dioctanoyl, adenosine, n6-cyclohexyl, salts of adenosine-5'-diphosphate, adenosine-5'-monophosphoric acid, adenosine-5'-o-(3-thiotriphosphate), salts of adenosine-5'-triphosphate, 9-beta-D-arabinoturanosyladenine, 1-beta-D-arabinoturanosylcytosine, 9-beta-D-arabinoturanosylguanine, 9-beta-D-arabinoturanosylguanine 5'-triphosphate, 1-beta-D-arabinoturanosylthymine, 5-azacytidine, 8-azaguanine, 3'-azido-3'-deoxythymidine, 6-benylaminopurine, cytidine phosphoramidite, beta-cyanoethyl diisopropyl, 249802cytidine-5'-triphosphate, 2'-deoxyadenosine, 2'-deoxyadenosine 5'-triphosphate, 2'-deoxycytidine, 2'-deoxycytidine 5'-triphosphate, 2'-deoxyguanosine, 2'-deoxyguanosine 5'-triphosphate, 2',3'-dideoxyadenosine, 2',3'-dideoxyadenosine 5'-triphosphate, 2',3'-dideoxycytidine, 2',3'-dideoxycytidine 5'-triphosphate, 2',3'-dideoxyguanosine, 2',3'-dideoxyguanosine 5'-triphosphate, 2',3'-dideoxyinosine, 2',3' dideoxythymidine, 2',3'-dideoxythymidine 5'-triphosphate, 2',3'-dideoxyuridine, N6-dimethylallyl adenine, 5-fluoro-2'-deoxyuridine, 5-fluorouracil, 5-fluorouridin, 5-fluorouridine 5'-monophosphate, formycin A 5'-triphosphate, formycin B, guanosine-3'-5'-cyclic monophosphate, guanosine-5'-diphosphate-3'-diphosphate, guanosine-5'-o-(2-thiotriphosphate), guanosine-5'-o-(3'-thiotriphosphate), guanosine 5'-triphosphate, 5'-guanylylimidodiphosphate, inosine, 5-iodo-2'-deoxyuridine, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotide phosphate, oligodeoxythymidylic acid, (p(dT)10), oligodeoxythymidylic acid (p(dT)12-18), polyadenylyc acid

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(poly A), polyadenylic acid-oligodeoxythymidynic acid, polycytidylic acid, poly(deoxyadenyl-deoxythymidylic acid, polydeoxyadenylic-acid-oligodeoxythymidynic acid, polydeoxythymidylic acid, polyinosine acid-polycytidylic acid, polyuridynic acid, ribonucleic acid, tetrahydro-uridine, thymidine, thymidine-3',5'-diphosphate, thymidine phosphoramidite, beta-cyanoethyl diisopropyl, 606102 thymidine 5'-triphosphate, thymine, thymine riboside, uracil, uridine, uridine-5'-diphosphoglucose, uridine 5'-triphosphate, xanthine, zeatine, transeatine riboside, etc. Further suitable polymers are: poly(DA) ss, poly(A) ss, poly(C) ss, poly(G) ss, poly(U) ss, poly(DA)-(DT) ds, complementary homopolymers, poly (D(A-T)) ds, copolymers, poly(DG)·(DC) ds, complementary homopolymers, poly (d(G-C)) ds copolymers, poly (d(L-C)) ds copolymers, poly(I)-poly(C) ds, etc. An oligopeptide or a polypeptide preferably contains 3-250, frequently 4-100, and very often 4-50 amino acids which are mutually coupled via amide-bonds. Suitable amino acids are usually of the alpha- and L-type; exceptions, however, such as in dermorphine are possible.

Peptides with a particularly high biological and/or therapeutic significance, and which can also be combined with transfersomes, are, for example, N-acetyl-Ala-Ala-Ala-, N-acetyl-Ala-Ala-Ala methyl ester, N-acetyl-Ala-Ala-Ala-Ala, N-acetyl-Asp-Glu, N-acetyl-Gly-Leu, N-alpha-Acetyl-Gly-Lys methyl ester acetate, acetyl-hirudine fragments, acetyl-5-hydroxy-Trp-5-hydroxy-Trp amide, des-acetyl-alpha-melanocyte stimulating hormone, N-Acetyl-Met-Asp-Arg-Val-Leu-Ser-Arg-Tyr, N-acetyl-Met-Leu-Phe, acetyl-muramyl-Ala-isoGln, N-acetyl-Phe-Tyr, N-acetyl-Phe-norLeu-Arg-Phe amide, N-acetyl-renine substrate tetradecapeptide, N-acetyl-transforming growth factor, adipokinetic hormone II, adjuvant peptide,

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adrenal peptide E, adrenocorticotrophic hormone (ACTH 1-39, Corticotropine A) and its fragments such as 1-4 (Ser-Tyr-Ser-Met), 1-10 (Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly), 1-17, 1-24 and 1-39, 11-24, 18-39, Ala-Ala, beta-Ala-Ala, Ala-Ala-Ala, Ala-Ala-Ala methyl ester, Ala-Ala-Ala-Ala, Ala-Ala-Ala-Ala-Ala, Ala-Ala-Ala-Ala-Ala-Ala, Ala-Ala-Phe, 7-amido-4-methylcoumarin, Ala-Ala-Phe p-nitroanilide, Ala-Ala-Val-Ala p-nitroanilide, Ala-Arg-Pro-Gly-Tyr-Leu-Ala-Phe-Pro-Arg-Met amide, beta-Ala-Arg-Ser-Ala-Pro-Thr-Pro-Met-Ser-Pro-Tyr, Ala-Asn, Ala-Asp, Ala-Glu, Ala-gamma-Gln-Lys-Ala-Ala, Ala-Gly, beta-Ala-Gly, Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Tyr-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe amide, Ala-Gly-Gly, Ala-Gly-Ser-Glu, Ala-His, beta-Ala-His, Ala-isoGln-Lys-Ala-Ala, Ala-Ile, Ala-Leu, beta-Ala-Leu, Ala-Leu-Ala, Ala-Leu-Ala-Leu, Ala-Leu-Gly, Ala-Lys, beta-Ala-Lys, Ala-Met, N-beta-Ala-1-methyl-His, Ala-norVal, Ala-Phe, beta-Ala-Phe, Ala-Phe-Lys 7-amido-4-methylcoumarin, Ala-Pro, Ala-Pro-Gly, Ala-sarcosine, Ala-Ser, Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr, Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr amide, Ala-Thr, Ala-Trp, beta-Ala-Trp, Ala-Tyr, Ala-Val, beta-Ala-Val, beta-Ala-Trp-Met-Asp-Phe amide, alytesine, amanitine, amastatine, angiotensine I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), II II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), III and related peptides, angiotensine II antagonist, angiotensine II receptor binding protein, angiotensine converting enzyme and its inhibitor (e.g. entipaine, bestatine, chymostatine, E-64, elastatinal, etc.) anserine, antide, aprotinine, arginine, vasopressine-Ala-Gly, Arg-Ala, Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly, Arg-Asp, Arg-Glu, Arg-Gly, Arg-Gly-Asp, Arg-Gly-Asp-Ser, Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro, Arg-Gly-Glu-Ser, Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala, Arg-His-Phe, Arg-Ile, Arg-Leu, Arg-Lys, Arg-Lys-Asp-Val-Tyr, Arg-Phe, Arg-Phe-Asp-Ser, Arg-Pro-Pro-Gly-Phe-Ser-

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Also worth mentioning are the collagen peptides, conicostatine, conicotropine releasing factor, conotoxin G1, M1, and GVIA, corticotropine-like intermediate lobe peptide, corticotropine releasing factor and related peptides, C-peptide, Tyr-C-peptide, cyclic calcitonine gene related peptides, cyclo(His-Phe-), cyclo(His-Pro-), cyclo(Leu-Gly-), cyclo(Pro-Gly-), Cys-Asp-Pro-Gly-Tyr-Ile-Ser-Arg amide, Cys-Gln-Asp-Ser-Glu-Thr-Arg-Thr-Phe-Tyr, DAGO, Delta-sleep inducing peptide, dermorphine,

(Ser(Ac)7)-dermorphine, diabetes associated peptide and its amide, N-alpha,N-epsilon-diacetyl-Lys-Ala-Ala, N-2,4-dinitrophenyl-Pro-Gln-Gly-Ile-Ile-Gly-Gln-Arg, diprotine A, dynorphines such as dynorphine A (Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Ser-Gln), fragments 1-6 (leucine enkephaline-Arg), 1-8, 1-13 or E-64, dynorphine B, ebelactones (e.g. A and B) ecarine, elastatinal, eledoisine and related peptides, alpha-, beta- and gamma-endorphine, endothelins, endorphines (e.g. alpha (=beta-Lipotropine 61-76), (Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr), beta (=beta-Lipotropine 61-91) and other beta-lipotropine-fragments, enkephaline and Leu-enkephaline (Tyr-Gly-Gly-Phe-Leu) and related peptides, enkephalinase inhibitors (e.g. epiamastatine, epibestatine, foroxymithine, leupeptine, pepstatine, Nle-Sta-Ala-Sta), eosinophilotactic tetrapeptide, epiamastatine, epibestatine, (Cys(Acm)20,31)-epidermal growth factor and its fragments or receptors, epidermal mitosis inhibiting pentapeptide, trans-epoxysuccinyl-Leu amido-(4-guanidino)butane, erythropoietine and fragment, S-ethylglutathione, fibrinogen related peptide, fibrinopeptide A and B, Tyr-fibrinopeptide A, (Glu1)-fibrinopeptide S, fibrinopeptide B-Tyr, fibroblast growth factor fragment 1-11, follicular gonadotropine releasing peptide, N-formylated peptides, foroxymithine, N-(3(2-furyl)acryloyl) peptide derivatives, galanine, GAP 1-13, gastric inhibitory polypeptide, gastrin related peptides and derivatives, gastrin releasing peptide, gastrointestinal peptides (e.g. Ala-Trp-Met-Asp-Phe-Amid, bombesin, caerulein, cholecystokinin, gelanin, gastrin, glucagon, motilin, neuropeptide K, pancreatic polypeptide, pancreaticozymine, PHI-27, secretin, valosin, etc.), Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys, (des-His1, Glu9)-glucagon amide, glucagon (1-37),

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glucagon-like peptide I, alpha-Glu-Ala, Glu-Ala-Glu, Glu-Ala-Glu-Asn, alpha-Glu-Glu, gamma-Glu-Glu, gamma-Glu-Gln, gamma-Glu-Gly, PGlu-Gly-Arg-Phe amide, alpha-Glu-Gly-Phe, gamma-Glu-His, gamma-Glu-Leu, alphaGlu-alpha-Lys, gamma-Glu-epsilon-Lys, N-gamma-Glu-Phe, PGlu-Ser-Leu-Arg-Trp amide, alpha-Glu-Trp, gamma-Glu-Trp, gamma-Glu-Tyr, alpha-Glu-Val, gamma-Glu-Val, PGlu-Val-Asn-Phe-Ser-Pro-Gly-Trp-Gly-Thr amide, A-Glu-Val-Phe, glutathiones and related peptides, glutathionesulfonic acid, Gly-Ala, Gly-beta-Ala, Gly-Ala-Ala, Gly-Ala-Ala-Ala-Ala, Gly-Ala-Tyr, Gly-alpha-aminobutyric acid, Gly-gamma-aminobutyric acid, Gly-Arg-Ala-Asp-Ser-Pro-Lys, Gly-Arg-Ala-Asp-Ser-Pro-OH, Gly-Arg-Gly-Asp-Ser, Gly-Arg-Gly-Asp-Asn-Pro-OH, Gly-Arg-Gly-Asp-Ser-OH, Gly-Arg-Gly-Asp-Ser-Pro-Lys, Gly-Arg-Gly-Asp-Ser-Pro-OH, Gly-Arg-Gly-Asp-Thr-Pro, Gly-Arg-Gly-Asp-Thr-Pro-OH, Gly-Arg p-nitroanilide, Gly-Arg-Gly-Asp, Gly-Arg-Gly-Asp-Ser, Gly-Asn, Gly-Asp, Gly-Asp-Asp-Asp-Asp-Lys, Gly-Glu, Gly-Gly and their derivatives such as methyl, ethyl or benzyl esters or amides, Gly-Gly-Ala, Gly-Gly-Arg, Gly-Gly-Gly, Gly-Gly-Gly-Gly, Gly-Gly-Gly-Gly-Gly, Gly-Gly-Gly-Gly-Gly-Gly, Gly-Gly-Ile, Gly-Gly-Leu, Gly-Gly-Phe, Gly-Gly-Phe-Leu, Gly-Gly-Phe-Leu amide, Gly-Gly-Phe-Met, Gly-Gly-Phe-Met amide, Gly-Gly-sarcosine, Gly-Gly-Tyr-Arg, Gly-Gly-Val, Gly-His, Gly-His-Arg-Pro, Gly-His-Gly, Gly-His-Lys, Gly-His-Lys-OH, Gly-Ile, Gly-Leu amide, Gly-Leu, Gly-Leu-Ala, Gly-Leu-Phe, Gly-Leu-Tyr, Gly-Lys, Gly-Met, Gly-norLeu, Gly-norVal, Gly-Phe amide, Gly-Phe, Gly-Phe-Ala, Gly-Phe-Arg, Gly-Phe-Leu, Gly-Phe-Phe, Gly-Pro, Gly-Pro-Ala, Gly-Pro-Arg, Gly-Pro-Arg-Pro, Gly-Pro-Arg-Pro-OH, Gly-Pro-Gly-Gly, Gly-Pro-hydroxy-Pro, Gly-sarcosine, Gly-Ser, Gly-Ser-Phe, Gly-Thr, Gly-Trp, Gly-Tyr amide, Gly-Tyr, Gly-Tyr-Ala, Gly-Val, Gly-Phe-Ser, granuliberine R, growth hormone releasing factor and its fragments, Hexa-Ala, Hexa-Gly, Hippuryl-Arg (Hip-Arg), Hippuryl-Gly-Gly (Hip-

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Gly-Gly), Hippuryl-His-Leu (Hip-His-Leu), Hippuryl-Lys, Hippuryl-Phe, hirudine and its fragments, His-Ala, His-Gly, His-Leu, His-Leu-Gly-Leu-Ala-Arg, His-Lys, His-Phe, His-Ser, His-Tyr, HIV envelope protein (gp120), Hydra peptides, P-hydroxyhippuryl-His-Leu, hypercalcemia malignancy factor (1-40), insulin chains B and C, P-iodo-Phe, Ile-Asn, Ile-Pro-Ile, insulin-like growth factor I (especially fragment 1-70), insulin-like growth factor II (especially its fragment 33-40), interleukin-1B fragment 163-171, isotocine, kassinine (Asp-Val-Pro-Lys-Ser-Asp-AGly-n-Phe-Val-Gly-Leu-Met-NH₂) katalcalcine (calcitonine precursor peptide), Tyr-katalcalcine, kemptide, kentsine, kyotorphine, laminine nonapeptide, laminine pentapeptide, laminine pentapeptide amide, leucine encephaline and related peptides, leucopyrokinine, Leu-Ala, Leu-beta-Ala, Leu-Arg, Leu-Asn, leucokinin I (Asp-Pro-Ala-Phe-Asn-Ser-Trp-Gly-NH₂) and II, Leucine-encephaline amide (Leu-encephaline amide) and related peptides, Leu-Gly, Leu-Gly-Gly, Leu-Gly-Phe, Leu-Leu amide, Leu-Leu, Leu-Leu-Leu amide, Leu-Leu-Leu, Leu-Leu-Phe amide, Leu-Leu-Tyr, Leu-Lys-Lys-Phe-Asn-Ala-Arg-Arg-Lys-Leu-Lys-Gly-Ala-Ile-Leu-Thr-Thr-Met-Leu-Ala, Leu-Met, Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys, Leu-Phe, Leu-Pro, Leu-Pro-Pro-Ser-Arg, Leu-Ser, Leu-Ser-Phe, Leu-Trp, Leu-Tyr, Leu-Val, leucotriene, Leu-Leu methyl ester, leupeptin, Leu-Ser-p-nitro-Phe-Nle-Ala-Leu methyl ester, beta-lipotropin fragments, litorine, luteinizing hormone releasing hormone and related peptides, lymphocyte activating pentapeptide, Lys-Ala, Lys-Ala 7-amido-4-methylcoumarin, Lys-Asp, Lys-Cys-Thr-Cys-Cys-Ala, Lys-Glu-Glu-Ala-Glu, Lys-Gly, Lys-Leu, Lys-Lys, Lys-Met, Lys-Phe, Lys-Pro-Pro-Thr-Pro-Pro-Pro-Glu-Pro-Glu-Thr, Lys-Serum thymic factor, Lys-Trp-Lys, Lys-Tyr-Trp-Trp-Phe amide, Lys-Val, macrophage inhibitory peptide (Tuftsine

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fragment 1-3, Thr-Lys-Pro), magainine I and II, mast cell degranulating peptide, mastoparan, alpha-mating factor, Melanin-Concentrating Hormone, MCD peptide, alpha-, beta-, gamma-, and delta-melanocyte stimulating hormones and related peptides, melittin, mesotocin, Met-beta-Ala, Met-Asn-Tyr-Leu-Ala-Phe-Pro-Arg-Met amide, methionine-enkephalin and related peptides, Met-Ala, Met-Ala-Ser, Met-Asn, methionine-enkephalin (Met-enkephalin, Tyr-Gly-Gly-Phe-Met) and related peptides, methionine-enkephalin amide (Met-Enkephalin amide, Tyr-Gly-Gly-Phe-Met-NH₂) and related peptides, Met-Gln-Trp-Asn-Ser-Thr-Thr-Phe-His-Gln-Thr-Leu-Gln-Asp-Pro-Arg-Val-Arg-Gly-Leu-Tyr-Phe-Pro-Ala-Gly-Gly, Met-Glu, Met-Gly, Met-Leu, Met-Leu-Phe, Met-Lys, Met-Met, Metorphamide, Met-Phe, Met-Pro, Met-Ser, Met-Tyr-Phe amide, Met-Val, N-Methoxycarbonyl-Nle-Gly-Arg, P-nitroaniline, methoxysuccinyl-Ala-Ala-Pro-Val, methoxysuccinyl-Ala-Ala-Pro-Val 7-amido-4-methylcoumarin, Met-somatotropine, molluscan cardioexcitatory peptide, morphiceptin, (Val³)-morphiceptin, motilin, MSH-release inhibiting factor, myelin basic protein or its fragments, naphthylamide-derivatives of various peptides, beta-naphthyl-Ala-Cys-Tyr-Trp-Lys-Val-Cys-Thr amide, alpha-neoendorphin, beta-neoendorphin, alpha-neurokinin, neurokinin A, (substance K, neuromedin L) and B, neoendorphin (alpha: Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro, beta, etc.) neuromedin B, C, K, U8, U-25 etc., neurokinin A and B, neuropeptides K and Y, neurophysin I and II, neurotensin and related peptides, nitroanilide peptide derivatives, Nle-Sta-Ala-Sta, NorLeu-Arg-Phe amide, opioid peptides (e.g. adrenal peptide E, Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-amides, casein fragments, casomorphin, N-CBZ-Pro-D-Leu, dermorphin, kyotorphin, morphiceptin (Tyr-Pro-Phe-Pro-NH₂), morphamide (Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val, adrenorphin),

osteocalcin (esp. its fragment 7-19), oxytocine and related peptides, pancreastatine and its fragments, such as 33-49, pancreatic polypeptide, pancreozymin, parathyroid hormone or fragments thereof, especially 1-34 and 1-84, penta-Ala, penta-Gly, penta-Phe, pepstatin A, peptide YY, peptide T, phalloidin, Phe-Ala-Ala-p-nitro-Phe-Phe-Val-Leu 4-pyridylmethyl ester, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe amide, Phe-Ala, Phe-Gly, Phe-Gly-Gly, Phe-Gly-Gly-Phe, Phe-Gly-Phe-Gly, Phe-Leu amide, Phe-Leu, Phe-Leu-Arg-Phe amide, Phe-Leu-Glu-Glu-Ile, Phe-Leu-Glu-Glu-Leu, Phe-Leu-Glu-Glu-Val, Phe-Met, Phe-Met-Arg-Phe amide, Phe-Phe, Phe-Phe-Phe, Phe-Phe-Phe-Phe, Phe-Phe-Phe-Phe-Phe, Phe-Pro, Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg, Phe-Tyr, Phe-Val, PHI-27, PHM-27, phosphoramidone, physalaemine (pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH₂), preproencephaline fragment 128-140, pressinoic acid and related peptides, Pro-Asn, proctoline (Arg-Tyr-Leu-Pro-Thr), proencephaline, Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys, Pro-Ala, Pro-Arg 4-methoxy-beta-naphthylamide, Pro-Asp, proglumide, Pro-Gly, Pro-Gly-Gly, Pro-hydroxy-Pro, Pro-Ile, Pro-Leu, Pro-Leu-Gly amide, Pro-Met, Pro-Phe amide, Pro-Phe, Pro-Phe-Arg 7-amido-4-methylcoumarin, Pro-Phe-Gly-Lys, Pro-Trp, Pro-Tyr, Pro-Val, cyclic AMP dependent protein kinase and its inhibitors, PyroGlu-Ala-Glu, PyroGlu-Ala, PyroGlu-Ala-Glu, PyroGlu-Asn-Gly, PyroGlu-Gly-Arg p-nitroanilide, PyroGlu-His-Gly amide, PyroGlu-His-Gly, PyroGlu-His-Pro amide, PyroGlu-His-Pro, PyroGlu-Lys-Trp-Ala-Pro, ranatensine, renine substrate tetradecapeptide, N-(alpha-rhamnopyranosyloxy-hydroxyphosphinyl) Leu-Trp, sarcosyl-Pro-Arg p-nitroanilide, sauvagine, sleep-inducing peptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu), secretine and related peptides, Ser-Ile-Gly-Ser-Leu-Ala-Lys, Ser-Ser-Ser, serum thymic factor, Ser-Ala, Ser-beta-Ala, Ser-Asn, Ser-Asp, Ser-Asp-Gly-Arg-Gly, Ser-Glu, Ser-Gln, Ser-Gly,

Ser-His, Ser-Leu, Ser-Met, Ser-Phe, Ser-Ser-Ser, Ser-Tyr, sleep inducing peptide, somastotone and related peptides (e.g. cyclo(p-Trp-Lys-Trh-Phe-Pro-Phe), steroido-genesis activator polypeptide, substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂) and related peptides, N-succinyl-derivatives of various peptides, syndyphalin-20 (Tyr-D-Met(O)-Gly-Phe-ol), tentoxin, tetra-Ala, tetra-Gly, thiostrepton, DL-thiorphane (encephalinase inhibitor), Thr-beta-Ala, Thr-Asp, Thr-Leu, Thr-Lys-Pro-Arg, Thr-Ser, Thr-Ser-Lys, Thr-Tyr-Ser, Thr-Val-Leu, thymopoietin fragments, thymosin alpha1 and its fragments, thymus circulating factor, thyrocalicitonin, thyrotropin releasing hormone, tocinoic acid, tosylated peptides, transforming growth factors, Tri-Ala, Tri-Ala methyl ester, Trp-Ala, Trp-Ala-Trp-Phe amide, Trp-Glu, Trp-Gly, Trp-Gly-Gly, Trp-His-Trp-Leu-Gln-Leu, Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr, Trp-His-Trp-Leu-Ser-Phe-Ser-Lys-Gly-Glu-Pro-Met-Tyr, Trp-Leu, Trp-Met-Asp-Phe amide, Trp-norLeu-Arg-Phe amide, Trp-Phe, Trp-Trp, Trp-Tyr, Tuftsin (Thr-Lys-Pro-Arg) and its fragments, Tyr-Ala, Tyr-Ala-Gly, Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu, Tyr-Ala-Gly-N-methyl-Phe 2-hydroxyethylamide, Tyr-Ala-Phe-Met amide, Tyr-Arg, Tyr-atrioepetin II, Tyr-Glu, Tyr-Gly, Tyr-Gly-Ala-Val-Val-Asn-Asp-Leu, Tyr-Gly-Gly, Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Arg, Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val amide, Tyr-Gly-Trp-Phe-Phe amide, Tyr-Leu, Tyr-Phe, Tyr-Phe-Met-Arg-Phe amide, Tyr-Phe-Phe amide, Tyr-Pro-Leu-Gly amide, Tyr-Pro-Phe-Pro amide, Tyr-Pro-Val-Pro amide, Tyr-Thr-Gly-Leu-Phe-Thr, Tyr-Tyr-Phe amide, Tyr-Trp-Ala-Trp-Phe amide, Tyr-Trp-Ala-Trp-Phe methylamide, Tyr-Tyr-Leu, Tyr-Tyr-Phe, Tyr-Tyr-Tyr, Tyr-Tyr-Tyr methyl ester, Tyr-Tyr-Tyr-Tyr-Tyr-Tyr, Tyr-Val amide, Tyr-Val, Tyr-Val-Gly, Urodilatin, Urotensin II, Valosin, Val-Ala, Val-Ala p-nitroanilide, Val-Ala-Ala-Phe, Val-Asp, Val-Glu, Val-Gln, Val-Glu-Glu-Ala-Glu, Val-Glu-Ser-Ser-Lys,

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Extended polypeptides are normally called proteins, independent of their detailed conformation. In this description, this term denotes, by and large, an enzyme or a coenzyme, an adhesion- or a recognition molecule, such as a CAMP or an OMP or a lectin, a histocompatibility complex, such as MHC-I or MHC-II, or an immunoglobuline (antibody) - or any (bio)chemical or (molecular)genetic modification thereof. Particularly useful for the applications according to this invention are the (bio)chemical modifications in which individual proteins are substituted with apolar residues, such as an alkyl, acyl, alkenoyl, etc. chains; but this is not a stringent limitation.

Oxidoreductases, such as: alcohol dehydrogenase (1.1.1.1), alcohol dehydrogenase (NADP dependent) (1.1.1.2), glycerol dehydrogenase (1.1.1.6), glycerophosphate dehydrogenase (1.1.1.8), xylulose reductase (1.1.1.10), polyol dehydrogenase (1.1.1.14), sorbitol dehydrogenase (1.1.1.14), myo-inositol dehydrogenase (1.1.1.18), uridine 5'-diphosphoglucose dehydrogenase

(1.1.1.22), glyoxalate reductase (1.1.1.26), lactate dehydrogenase (1.1.1.27), lactate dehydrogenase (1.1.1.28), glycerate dehydrogenase (1.1.1.29), beta-hydroxybutyrate dehydrogenase (1.1.1.30), beta-hydroxyacyl CoA dehydrogenase (1.1.1.35), malate dehydrogenase (1.1.1.37), malate enzyme (1.1.1.40), isocitric dehydrogenase (1.1.1.42), 6-phosphogluconate dehydrogenase (1.1.1.44), glucose dehydrogenase (1.1.1.47), beta-galactose dehydrogenase (1.1.1.48), glucose-6-phosphate dehydrogenase (1.1.1.49), 3alpha-hydroxysteroid dehydrogenase (1.1.1.50), 3beta-hydroxysteroid dehydrogenase (1.1.1.51), 3alpha,2beta-hydroxysteroid dehydrogenase (1.1.1.53), 3-phosphoglycerate dehydrogenase (1.1.1.95), fucose dehydrogenase (1.1.1.122), lactate dehydrogenase (cytochrome) (1.1.2.3), glucose oxidase (1.1.3.4), cholesterol oxidase (1.1.3.6), galactose oxidase (1.1.3.9), alcohol oxidase (1.1.3.13), glycolate oxidase (1.1.3.15), choline oxidase (1.1.3.17), glycerol-3-phosphate oxidase (1.1.3.21), xanthine oxidase (1.1.3.22), alcohol dehydrogenase (1.1.99.8), fructose dehydrogenase (1.1.99.11), formaldehyde dehydrogenase (1.2.1.1), formate dehydrogenase (1.2.1.2), aldehyde dehydrogenase (1.2.1.5), glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12), gabase (1.2.1.16), pyruvate oxidase (1.2.3.3), oxalate oxidase (1.2.3.4), dihydroorotate dehydrogenase (1.3.3.1), lipoxidase (1.3.11.12), alanine dehydrogenase (1.4.1.1), glutamic dehydrogenase (1.4.1.3), glutamate dehydrogenase (NADP) (1.4.1.4), L-amino acid oxidase (1.4.3.2), D-amino acid oxidase (1.4.3.3), monoaminoxidase (1.4.3.4), diaminoxidase (1.4.3.6), dihydrofolate reductase (1.5.1.3), 5,10-methylenetetrahydrofolat dehydrogenase (1.5.1.5), saccharopine dehydrogenase NAD+ (1.5.1.7), octopine dehydrogenase (1.5.1.11), sarcosine oxidase (1.5.3.1),

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sarcosine dehydrogenase (1.5.99.1), glutathione reductase (1.6.4.2), ferridoxin-NADP+ reductase (1.6.7.1), NADPH-FMN oxidoreductase (1.6.99.1), cytochrome c reductase (1.6.99.3), NADH-fmn oxidoreductase (1.6.99.3), dihydropteridin reductase (1.6.99.7), uricase (1.7.3.3), diaphorase (1.8.1.4), lipoamide dehydrogenase (1.8.1.4), cytochrome oxidase (1.9.3.1), nitrate reductase (1.9.6.1), phenolase (1.10.3.1), ceruloplasmine (1.10.3.2), ascorbate oxidase (1.10.3.3), NADH peroxidase (1.11.1.1), catalase (1.11.1.6), lactoperoxidase (1.11.1.7), myeloperoxidase (1.11.1.7), peroxidase (1.11.1.7), glutathione peroxidase (1.11.1.9), chloroperoxidase (1.11.1.10), lipoxidase (1.13.1.12), protocatechuate 3,4-dioxygenase (1.13.11.3), luciferase (glow-worm) (1.13.12.7), salicylate hydroxylase (1.14.13.7), p-hydroxybenzoate hydroxylase (1.14.13.2), luciferase (bacterial) (1.14.14.3), phenylalanine hydroxylase (1.14.16.1), dopamine-beta-hydroxylase (1.14.17.1), tyrosinase (1.14.18.1), superoxide dismutase (1.15.1.1), ferredoxine-NADP reductase (1.18.1.2), etc.. Transferases, such as: catecholic o-methyltransferase (2.1.1.6), phenylethanol-amine N-methyl-transferase (2.1.1.28), aspartate transcarbamylase (2.1.3.2), ornithine carbamyltransferase (2.1.3.3), transketolase (2.2.1.1), transaldolase (2.2.1.2), choline acetyltransferase (2.3.1.6), carnitine acetyltransferase (2.3.1.7), phosphotransacetylase (2.3.1.8), chloroamphenicol acetyltransferase (2.3.1.28), kanamycine 6'-acetyltransferase (2.3.1.55), gentamicine acetyltransferase (2.3.1.60), transglutaminase (2.3.2.13), gamma-glutamyl transpeptidase (2.3.2.2), phosphorylase A (2.4.1.1), phosphorylase B (2.4.1.1), dextranucrase (2.4.1.5), sucrose phosphatase (2.4.1.7), glycogen synthase (2.4.1.11), uridine 6'-diphospho-glucuronyltransferase (2.4.1.17), galactosyl trans-

Transpeptidases, such as: esterase (3.1.1.1), lipase (3.1.1.3), phospholipase A (3.1.1.4), acetyl esterase (3.1.1.6), cholinesterase, acetyl (3.1.1.7), choline-esterase, butyryl (3.1.1.8), pectinesterase (3.1.1.11), cholesterol esterase (3.1.1.13), glyoxalase ii (3.1.2.6), phosphatase, alkaline (3.1.3.1), phosphatase acid (3.1.3.2), 5'-nucleotidase (3.1.3.5), 3'-nucleotidase (3.1.3.6), glucose-6-phosphatase (3.1.3.9), fructose-1,6-diphosphatase (3.1.3.11), phytase (3.1.3.26), phosphodiesterase i (3.1.4.1), glycerophosphorylcholine (3.1.4.2), phospholipase C (3.1.4.3), phospholipase D (3.1.4.4), deoxyribonuclease I (3.1.4.5), deoxyribonuclease II (3.1.4.6), ribonuclease N1 (3.1.4.8), sphingomyelinase (3.1.4.12), phosphodiesterase 3':5'-cyclic (3.1.4.17), phosphodiesterase II (3.1.4.18), endonuclease (3.1.4.21), ribonuclease A (3.1.4.22), ribonuclease B (3.1.4.22), 3'-phosphodiesterase 2':3'-cyclic nucleotide (3.1.4.37), sulfatase (3.1.6.1), chondro-4-sulfatase (3.1.6.9),

chondro-6-sulfatase (3.1.6.10), ribonuclease T2 (3.1.27.1), ribonuclease T1 (3.1.27.3), ribonuclease u2 (3.1.27.4), nuclease (3.1.30.1), nuclease, (from micrococces) (3.1.31.1), alpha-amylase (3.2.1.1), beta-amylase (3.2.1.2), amyloglucosidase (3.2.1.3), cellulase (3.2.1.4), laminarinase (3.2.1.6), dextranase (3.2.1.11), chitinase (3.2.1.14), pectinase (3.2.1.15), lysozyme (3.2.1.17), neuraminidase (3.2.1.18), alpha-glucosidase, maltase (3.2.1.20), beta-glucosidase (3.2.1.21), alpha-galactosidase (3.2.1.22), beta-galactosidase (3.2.1.23), alpha-mannosidase (3.2.1.24), beta-mannosidase (3.2.1.25), invertase (3.2.1.26), trehalase (3.2.1.28), beta-N-acetylglucosaminidase (3.2.1.30), beta-glucuronidase (3.2.1.31), hyaluronidase (3.2.1.35), beta-xylosidase (3.2.1.37), hesperidinase (3.2.1.40), pullulanase (3.2.1.41), alpha-fucosidase (3.2.1.51), mycodextranase (3.2.1.61), agarase (3.2.1.81), endoglycosidase F (3.2.1.96), endo-alpha-N-acetylgalactosaminidase (3.2.1.97), NADase (nicotinamide adenine glycopeptidase) F (3.2.2.5), dinucleotidase (3.2.2.18), thiogluc (3.2.3.1), s-adenosylhomocystein-hydrolase (3.3.1.1), leucin-aminopeptidase, (from cytosol) (3.4.11.1), leucin-aminopeptidase, microsomale (3.4.11.2), pyroglutamate-aminopeptidase (3.4.11.8), carboxypeptidase a (3.4.12.2), carboxypeptidase B (3.4.12.3), prolidase (3.4.13.9), cathepsin C (3.4.14.1), carboxypeptidase W (3.4.16.1), carboxypeptidase A (3.4.17.1), carboxypeptidase B (3.4.17.2), alpha-chymotrypsin (3.4.21.1), beta-chymotrypsin (3.4.21.1), gamma-chymotrypsin (3.4.21.1), delta-chymotrypsin (3.4.21.1), trypsin (3.4.21.4), thrombin (3.4.21.5), plasmin (3.4.21.7), kallikrein (3.4.21.8), enterokinase (3.4.21.9), elastase from pancreas (3.4.21.11), protease (subtilisin) (3.4.21.14), urokinase (3.4.21.31), elastase from leucocytes (3.4.21.37), cathepsin B, (3.4.22.1), papain (3.4.22.2),

ficin (3.4.22.3), bromo-elain (3.4.22.4), chymopapain (3.4.22.6), clostripain (3.4.22.8), proteinase A (3.4.22.9), pepsine (3.4.23.1), renine (3.4.23.4), cathepsin D (3.4.23.5), protease (aspergillopeptidase) (3.4.23.6), collagenase (3.4.24.3), collagenase (3.4.24.8), pinguinain (3.4.99.18), renine (3.4.99.19), urokinase (3.4.99.26), asparaginase (3.5.1.1), glutaminase (3.5.1.2), urease (3.5.1.5), acylase i (3.5.1.14), cholyglycine hydrolase (3.5.1.24), urease(ATP-hydrolyzing) (3.5.1.45), penicillinase (3.5.2.6), cephalosporinase (3.5.2.8), creatininase (3.5.2.10), arginase (3.5.3.1), creatinase (3.5.3.3), guanase (3.5.4.3), adenosine-deaminase (3.5.4.4), 5'-adenylate acid-deaminase (3.5.4.6), creatinine deiminase (3.5.4.21), anorganic pyrophosphatase (3.6.1.1), adenosine 5'-triphosphatase (3.6.1.3), apyrase (3.6.1.5), pyrophosphatase, nucleotide (3.6.1.9), etc.

Lyases, such as: pyruvate-decarboxylase (4.1.1.1), oxalate decarboxylase (4.1.1.2), oxalacetate decarboxylase (4.1.1.3), glutamic decarboxylase (4.1.1.15), ornithine decarboxylase (4.1.1.17), lysine decarboxylase (4.1.1.18), arginin decarboxylase (4.1.1.19), histidine decarboxylase (4.1.1.22), orotidine 5'-monophosphate decarboxylase (4.1.1.23), tyrosine decarboxylase (4.1.1.25), phospho(enol) pyruvate carboxylase (4.1.1.31), ribulose-1,5-diphosphate carboxylase (4.1.1.39), phenylalanine decarboxylase (4.1.1.53), hydroxymandelonitrilelyase (4.1.2.11), aldolase (4.1.2.13), N-acetylneuramine acid aldolase (4.1.3.3), etc. citrate lyase (4.1.3.6), citrate synthase (4.1.3.7), tryptophanase (4.1.99.1), isozymes of carbonic anhydrase (4.2.1.1), fumarase (4.2.1.2), aconitase (4.2.1.3), enolase (4.2.1.11), crotonase (4.2.1.17), delta-amino-levulinate dehydratase (4.2.1.24), chondroitinase ABC

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The following are, amongst others, referred to as proteases: aminopeptidase M, amino acid-arylamidase, bromo-elaine, carboxypeptidase A, carboxypeptidase B, carboxypeptidase P, carboxypeptidase Y, cathepsine C, chymotrypsine, collagenases, collagenase/dispase, dispase, elastase, endoproteinase Arg-c, endoproteinase Asp-n sequencing grade, endoproteinase Glu-c (proteinase V8), endoproteinase Glu-c sequencing grade, endoproteinase Lys-c, endoproteinase Lys-c sequencing grade, endoproteinses, factor Xa, ficine, kallikrein, leucine-aminopeptidase, papaine, pepsine, plasmin, pronase, proteinase K, proteinase V8 (endoproteinase Glu-c), pyroglutamate-aminopeptidase, pyroglutamate-aminopeptidase, restriction protease factor Xa, subtilisine, thermolysine, thrombine, trypsin, etc.

A coenzyme according to this invention is any substance which supports enzyme activity. Amongst the biologically important coenzymes are, for example, acetyl-coenzyme A,

Another class of proteins, which are important in the context of this invention, are lectins. Plants, and sometimes also animal, tissues are suitable sources of lectins; particularly convenient sources are *Abrus precatorius*, *Agaricus bisporus*, *Agrostemma githago*, *Anguilla anguilla*, *Arachis hypogaea*, *Artocarpus integrifolia*, *Bandeiraea simplicifolia* BS-I und BS-II, (*Griffonia simplicifolia*), *Banhlula purpurea*, *Caragana arborescens*, *Cicer arietinum*, *Canavalia ensiformis* (jack bean), *Caragana arborescens* (Siberian pea tree), *Codium fragile* (green algae), *Concanavalin A* (Con A), *Cytisus scoparius*, *Datura stramonium*, *Dolichos biflorus*, *Erythrina corallodendron*, *Euonymus europaeus*, *Gelonium multiflorum*, *Glycine max* (soy), *Griffonia simplicifolia*, *Helix aspersa* (garden snail), *Helix pomatia* (escargot), *Laburnum alpinum*, *Lathyrus odoratus*, *Lens culinaris* (lentil), *Limulus polyphemus*, *Lycopersicon esculentum* (tomato), *Lotus tetragonolobus*, *Luffa aegyptiaca*, *Maclura pomifera* (Osage orange), *Momordica charantia* (bitter pear melon), *Naja mocambique* (Mozambiquan cobra), *Naja Naja kaouthia*, *Mycoplasma gallisepticum*, *Perseu americana* (avocado), *Phaseolus coccineus* (beans), *Phaseolus limensis*, *Phaseolus lunatus*, *Phaseolus vulgaris*, *Phytolacca americana*, *Pseudomonas aeruginosa* PA-I, *Pisum sativum* (pea), *Ptilota plumosa* (red algae), *Psophocarpus tetragonolobus* (winged bean), *Ricinus communis* (castor bean), *Robinia pseudoacacia* (false acacia, black locust), *Sambucus nigra* (clematis), *Saponaria officinalis*, *Solanum tuberosum* (potato), *Sophora japonica*, *Tetragonolobus purpureas* (winged or asparagus pea), (*Lotus tetragono-*

lobus), *Tritigum vulgare* (wheat germ), *Ulex europaeus*,
Vicia faba, *Vicia sativa*, *Vicia villosa*, *Vigna radiata*,
Viscum album (mistle), *Wisteria floribunda*, etc.

Further interesting proteins are, e.g. the activator of tissue-plasminogen, insulin, kallikrein, keratin, kininogene, lactoterrin, laminarin, laminin, alpha2-macroglobuline, alpha1-microglobuline, F2-microglobuline, high density lipoproteins, basic myeline-protein, myoglobine, neurofilaments I, II, and III, neurotensine, oxytocine, pancreatic oncofoetal antigen, parvalbumin, plasminogen, platelet factor 4, pokeweed antiviral protein, porphobilinogen, prealbumin, prostate specific antigens, protamine sulfate, protein C, protein C activator, protein S, prothrombin, retinol binding protein, S-100 protein, pregnancy protein-1, serum amyloid A, serum amyloid P component, tenascine, testosterone-estradiol binding globuline, thioredoxine, thrombine, thrombocyte, beta-thromboglobuline, thromboplastine, microsomal antigen from thyroidea, thyroidea stimulating hormone, thyroxine binding globuline, transcortine, transferrine, ubiquitine, vimentine, vinculine, vitronectine, etc.

Some typical examples of human and animal hormones which can be used as agents according to the invention are, for example, acetylcholine, adrenaline, adrenocorticotrophic hormone, angiotensine, antidiuretic hormone, cholecystokinin, chorionic gonadotropine, corticotropine A, danazol, diethylstilbestrol, diethylstilbestrol glucuronide, 13,14-dihydro-15-keto-prostaglandins, 1-(3',4'-dihydroxyphenyl)-2-aminoethanol, 5,6-dihydroxy-tryptamine, epinephrine, follicle stimulating hormone, gastrin, gonadotropin, β -hypophamine, insulin, juvenile hormone, 6-ketoprostaglandins, 15-ketoprostaglandins,

LTH, luteinizing hormone releasing hormone, luteotropic hormone, α -melanocyte stimulating hormone, gamma-melanocyte stimulating hormone, 5-melanocyte stimulating hormone, noradrenaline, norepinephrine, oxytocine, parathyroid hormone, parathyroid substances, prolactine, prostaglandins, secretine, somatostatine, somatotropine (STH), thymosine alpha 1, thyrocalcitonine, thyroglobuline, thyroid stimulating hormone, thyrotropic hormone, thyrotropine releasing hormone, 3,3',5-triiodothyroacetic acid, 3,3',5'-triiodothyronine, TSH, vasopressine, etc.

Oestrogens are mostly steroid hormones with 18 carbon atoms and one unsaturated (aromatic) ring. Amongst the most important oestrogens are, for example, chlorotrianisene, diencestrole, diethylstilboestrole, diethylstilboestrol-dipropionate, diethylstilboestrol-disulfate, dimestrole, estradiol, estradiolbenzoate, estradiolundecylate, estriolsuccinate, estrone, ethinglestradiol, nexoestrole, nestranole, oestradiolvalerate, oestriole and quineestrole.

Gestagenes are typically synthetic hormones, mainly with progesterone-like characteristics; the most important agents belonging to this class are allylestrenole, chloromadinonacetate, dimethisterone, ethisterone, hydroxyprogesteron-caproate, lynestrenole, medrogestone, medroxyprogesteron-acetate, megestrolacetate, methyloestrenolone, norethisterone, norethisterone-acetate, and norgestrel.

Agents can also be parts of a biological extract. As sources of biologically and/or pharmacologically active extracts, the following are worth-mentioning: for example, *Acetobacter pasteurianum*, *Acokanthera ouabaio*

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cathel, Aesculus hippocastanum, Ammi visnaga Lam., Ampi Huasca, Apocynum Cannabium, Arthrobotrys superba var. oligospora (ATCC 11572), Atropa belladonna, Bacillus Lentus, Bacillus polymyxa, Bacillus sphaericus, Castilloa elastica cerv., Chondrodendron tomentosum (Ampi Huasca), Convallaria majalis, Coronilla-enzymes, Corynebacterium hoagii (ATCC 7005), Corynebacterium simplex, Curvularia lunata (Wakker) Boadijn, Cylindrocarpon radicola (ATCC 11011), Cynara scolymus, Datura Metel, didymella, digilanidase, digitalis Lanata, digitalis purpurea, Duboisia, Flavobacterium dehydrogenans, Fusarium exquiseti saccardo, Hyoscyamus niger, Jaborandi-leaves (P. microphyilus Stapf), Micromonosporapurpurea u. echinospora, Paecilomyces varioti Bainier var. antibioticus, Penicillium chrysogenum Thom, Penicillium notatum Westling, Penicillium patulum, Rauwolfia serpentina Benth., Rhizopus arrhizus Fischer (ATCC-11145), Saccharomyces cerevisiae, Schizomycetes ATCC-7063, Scilla maritima L., Scillarenase, Septomyxa affinis (ATCC 6737), Silybum marianum Gaertn., Streptomyces ambofaciens, Strophantusgratus, Strophantus Kombe, Thevetia peruviana, Vinca minor L., Vinca rosea, etc.

Unless stated otherwise, all substances, surfactants, lipids, agents or additives with one or several chiral carbon atoms can be used either as a racemic mixture or in the form of optically pure enantiomers.

WORKING PRINCIPLE

The transport of agents through permeation barriers can be mediated by such carriers which fulfill the following basic criteria:

- carriers should experience or create a gradient which drives them into or through a barrier, e.g. from the body surface into or through the skin, or from the surface of a leaf into the depth of a leaf, or from one side of a barrier to the other;
- the resistance to permeation which is felt by the carriers in the barrier should be as small as possible in comparison to the driving force;
- carriers should be capable of permeating in and/or through a barrier without thereby losing their associated agents in an uncontrollable manner.

Carriers, moreover, should preferably provide control of the distribution of agents, as well as over the effectiveness and temporal development of the agents action. They should be capable of bringing materials into the depth of and across a barrier, if so desired, and/or should be capable of catalyzing such a transport. Last but not least, such carriers should affect the range and depth of action as well as the type of cells, tissue parts, organs and or system parts which can be reached or treated, under suitable conditions at least.

In the first respect, chemical gradients are especially convenient for biological applications. Particularly suitable are the physico-chemical gradients, such as the pressure of (de)hydration pressure (humidity gradient) or a difference in concentration between the sites of application and action; however, electrical or magnetic fields as well as thermal gradients are also interesting in this respect. In technological applications, an externally applied pressure or existing hydrostatic pressure difference are also of importance.

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In order to fulfill the second condition, carriers must be sufficiently 'fluid' at the microscopic scale; this enables them to easily cross the constrictions in the permeability barrier.

Permeation resistance is a decreasing function of the decreasing carrier size. But also the carrier driving force frequently depends on the size of the permeating particle, droplet or vesicle; when the driving pressure is size-independent, the corresponding force also typically decreases with decreasing carrier size. This causes the transfer effectiveness to be a complex function of the carrier size, often showing a maximum depending on the chosen carrier and/or agent composition.

In the case of molecular aggregates the permeation resistance is largely determined by the mechanical elasticity and deformability of the carrier, the viscosity of the total preparation being also important, however. The former must be sufficiently high, the latter low enough.

Size and, even better, deformability can serve as a criterion for the optimization of the supramolecular carriers according to this invention. As an indication of deformability, the capacity of individual carriers to form protrusions can be studied, as a function of all relevant system parameters. (In practical terms, it is often sufficient to investigate only such variables which come into question for a controllable application. The examples given in this application, therefore, only pertain to varying the concentrations of the edge active components and the absolute carrier concentration which affect the forced diminishment of the lipid vesicle or of vesicle permeation.) This is true e.g. for transcutaneous and transcuticular transport as well as for the transport of agents through the lung alveoli, into the hair, into gels, and

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the like.

With regard to the third requirement, the choice of the carriers, agents and additives, as well as the applied carrier dose or concentration all play some role. Low dose, in the majority of cases, gives rise to a predominantly surface treatment: poorly water-soluble substances in such case remain confined largely to the apolar region of a permeability barrier (such as in the epidermal membranes); agents which are highly soluble and can diffuse easily from the carriers can attain a distribution which is different from that of the carrier particles; for such substances, the permeability of a transfersomal membrane is also important. Edge active substances with a tendency to leave carriers and move into a barrier give rise to a locally variable carrier composition, etc. These interdependencies should be thought of and considered prior to each individual application. In the search for a set of conditions under which a simple carrier vesicle becomes a transfersome, the following rules of thumb can be used:

- At first, the conditions are determined under which the carrier vesicles are solubilized by the edge active substances. At this critical point the 'vesicles' are maximally deformable owing to the fact that they are permanently formed and deformed. At the same time, however, they are also unstable and incapable of holding and transferring water soluble substances.
- Next, the carrier composition or concentration is adapted by reducing the edge activity in the system to an extent which ensures the vesicle stability as well vesicle deformability to be sufficiently high; this also ensures the permeation capacity of such carriers to be satisfactory. The term stability in this application implies,

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on the one hand, a mechanical tendency of the carrier components to "stay together"; on the other hand, that the carrier composition during the transport, and in particular during the permeation process, does not change at all or not much. The position of the corresponding optimum which one is looking for hereby depends on many boundary conditions. The type of agent molecules also plays an important role in this. The smaller and the more hydrophilic the agent to be transported, the further the carrier system must be spaced from the solubilization point; the desired shelf life of carriers is also important: upon approaching the solubilization point, the tendency of transfersomes to form larger particles may increase and the carrier's storage capacity simultaneously decrease.

- Ultimately, the system parameters need to be optimized with respect to the envisaged modes and goals of a given application. Rapid action requires a high permeation capability; in order to achieve slow drug release, it is advantageous to ensure gradual penetration through the permeability-barrier and a correspondingly 'finely adjusted' membrane permeability; in order to reach deep regions, high doses are needed; in order to obtain a broad distribution, it is recommended to use carrier concentrations which are not too high.

This application describes some relevant properties of the transfersomes as carriers for the lipid vesicles. Most of the examples pertain to carriers made of phospholipids, but the general validity of conclusions is not restricted to this carrier or molecule class. The vesicle examples should only illustrate the requirements which should be fulfilled in order to attain penetration through permeability barriers, such as skin. Similar properties, moreover, ensure carrier transport

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The fact that the cells in a horny skin layer continuously merge with the watery compartments of subcutis is probably one reason for the spontaneous permeation of transfersomes through the 'pores' in this layer: during the permeation process transfersomes are propelled by the osmotic pressure. As an alternative, external pressures, such as an electroosmotic or hydrostatic pressure, however, can also be applied in addition.

It is sometimes convenient to adjust the pH-value of a formulation immediately after it has been prepared or directly prior to an application. Such an adjustment should prevent the deterioration of individual system components and/or drug carriers under the conditions of initial pH; simultaneously, a physiological compatibility should be achieved. For the neutralization of carrier suspensions, physiologically tolerable acids or bases are most frequently used as well as buffers with a pH-value between 3-12, preferably 5 to 9 and most often 6-8, depending on the goal and site of application. Physiologically acceptable acids are, for example, diluted aqueous solutions of mineral acids, such as hydrochloric acid, sulfuric acid, or phosphoric acid, or organic acids, such as carboxyalkane acids, e.g. acetic acid. Physiologically acceptable bases are, for example, diluted sodium hydroxide,

suitably ionized phosphoric acids, etc.

Formulation temperature is normally chosen to be well suited for the given substances; for aqueous preparations it is normally in the range of 0 to 95°C. Whenever possible, one should work in the temperature range 18-70°C; particularly preferred are temperatures between 15 and 55°C for the work with fluid chain lipids; the preferred temperature range for the lipids with ordered chains is from 45 to 60°C. Other temperature ranges are possible, however, most notably for the non-aqueous systems or preparations containing cryo- or heat-stabilizers.

If required by the sensitivity of one of the system components, transfersome formulations can be stored in cold (e.g. at 4°C). It is, moreover, possible to make and keep them under an inert atmosphere, e.g. under nitrogen. Shelf-life, furthermore, can be extended if no substances with multiple bonds are used, and if the formulation is (freeze) dried, or if a kit of dry starting materials is dissolved or suspended and processed at the site of application only.

In the majority of cases, carriers are applied at room temperature. But applications at lower or higher temperatures are also possible, especially when synthetic substances are used.

Transfersomal preparations can be processed previously or at the site of application, as has been described, for example, in our previous German patent application P 40 26 833.0-43, and exemplified in several cases in the handbook on 'Liposomes' (Gregoriadis, G., Edits. CRC Press, Boca Raton, Fl., Vols 1-3, 1987), in the monography 'Liposomes as drug carriers' (Gregoriadis, G., Edits. John Wiley & Sons, New York, 1988), or in the laboratory manual 'Liposomes. A Practical Approach' (New, R., Oxford-Press, 1989). If

required any suspension of drugs, moreover, can be diluted or concentrated (e.g. by per ultracentrifugation or ultrafiltration) immediately prior to a final application; additives can also be given into a preparation at this or a previous time. Upon any such manipulation, however, a possible shift of the permeation optimum for a given carrier preparation must be taken into account or prevented.

Transfersomes as described in this applications are well suited to be used as carriers of lipophilic substances, such as fat-soluble biological agents, therapeutics, poisons, etc. But it is quite likely that transfersomes used in combination with water soluble substances, especially when the molecular weight of the latter exceeds 1000 Dt, will be of even greater practical value.

Transfersomes, moreover, can contribute to the stabilization of substances which are sensitive to hydrolysis; they can improve carrier and drug distribution in the specimen and at the site of application and can also ensure a more favourable effect of the drug in time. Basic carrier ingredients can also bring advantages of their own. However, the most important carrier characteristics is the capability of transporting materials into and through a permeability barrier; this opens up a way for applications which prior to this discovery were not feasible.

The specific formulations as described in this invention have been optimized for the topical use on - or in the vicinity of - (a) permeability barrier(s). Particularly interesting barriers of this kind are skin and plant cuticle. (But formulations according to this invention are also well suited for the peroral (p.o) or parenteral (i.v. i.m. or i.p.) application, especially when edge active substances have been chosen in order to keep the drug loss at the site of

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application low.) Edge active substances which have a diminished activity, are degraded preferentially, are absorbed particularly efficiently or are diluted strongly at the site of application are especially valuable in this last respect.

In dermatology, application doses of up to 50, often up to 10 and very frequently less than 2.5 (or even less than 1 mg) of carrier substance are used per cm^2 of skin surface, the given masses pertaining to the basic carrier substance. The optimal mass depends on the carrier composition, desired penetration depth and duration of action, as well as on the detailed application site. Application doses useful in agrotechnics are typically lower and frequently below 0.1g pro m^2 .

Depending on the goal of application, each formulation can also contain suitable solvents up to a total concentration which is determined by certain plausible physical (no solubilization or appreciable shift of penetration optimum), chemical (no lowering of stability), or biological and physiological (little adversary side effects) formulation requirements.

Quite suitable for this purpose are, for example, the unsubstituted or substituted, e.g. halogenated, aliphatic, cycloaliphatic, aromatic or aromatic-aliphatic hydrocarbons, such as benzol, toluol, methylene chloride or chloroform, alcohols, such as methanol or ethanol, propanediol, erithritol, short-chain alkane carboxylic acid esters, such as acetic acid acid alkylesters, such as diethylether, dioxan or tetrahydrofuran, or mixtures thereof.

A survey of the lipids and phospholipids which can be used for the applications as described in this report in addition to the ones already mentioned is given, for example, in 'Form and

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function of phospholipids' (Ansell & Hawthorne & Dawson, eds.), 'An Introduction to the Chemistry and Biochemistry of Fatty Acids and Their Glycerides' of Gunstone and in other reference books. All implicitly and explicitly mentioned lipids and surfactants as well as other suitable edge active substances and their preparation are well known. A survey of available surfactants, together with the trademarks under which they are marketed by their manufacturers, is given in the annals 'Mc Cutcheon's, Emulsifiers & Detergents', Manufacturing Confectioner Publishing Co. An up-to-date compilation of the pharmaceutically acceptable agents is given, for example, in 'Deutsches Arzneibuch' (and in the annually updated list 'Rote Liste'); furthermore, in the British Pharmaceutical Codex, European Pharmacopoeia, Farmacopoeia Ufficiale della Repubblica Italiana, Japanese Pharmacopoeia, Nederlandse Pharmacopoeia, Pharmacopoeia Helvetica, Pharmacopée Française, The United States Pharmacopoeia, The United States NF, etc. A concise list of suitable enzymes can be found in the volume on 'Enzymes', 3rd Edition (M. Dixon and E.C. Webb, Academic Press, San Diego, 1979); more recent developments are described in the series 'Methods in Enzymology'. Many examples of the glycohydate-binding proteins which could be interesting for the use in combination with carriers as described in this invention are quoted in 'The Lectins: Properties, Functions, and Applications in Biology and Medicine' (I.E. Liener, N. Sharon, I.T. Goldstein, Eds. Academic Press, Orlando, 1986) as well as in the corresponding special publications; substances which are particularly interesting for agrotechnical applications are described, for example, in 'The Pesticide Manual' (C.R. Worthing, S.B. Walker, Eds. British Crop Protection Council, Worcestershire, Englande, 1986, e.g. 8th edition) and in 'Wirkstoffe in Pflanzenschutz und Schädlingsbekämpfung', which is published by Industrie-Verband Agrar (Frankfurt); most commonly available antibodies are listed in the catalogue

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'Linscott's Directory', the most important neuropeptides in 'Brain Peptides' (D.T. Krieger, M.J. Brownstein, J.B. Martin, Eds. John Wiley, New York, 1983), corresponding supplementary volumes (e.g. 1987) and other special journals.

Methods for the preparation of liposomes, which in the majority of cases can also be used for manufacturing transfersomes, are described, for example, in 'Liposome Technology' (Gregoriadis, Ed., CRC Press) or older books dealing with similar topics, such as 'Liposomes in Immunobiology' (Tom & Six, Eds., Elsevier), 'Liposomes in Biological Systems' (Gregoriadis & Allison, Eds., Willey), 'Targeting of Drugs' (Gregoriadis & Senior & Trouet, Plenum), etc. Corresponding patent publications also are a valuable source of relevant information.

The following examples are aimed at illustrating this invention without restricting it. All temperatures are in degrees Celsius, carrier sizes in nanometers, pressures in Pascal and other units in standard SI system.

Ratios and percentages are given in moles, unless otherwise stated.

Examples 1-13:

Composition:

250-372 mg	phosphatidylcholine from soy-bean (+95 % = PC)
187-34.9 mg	oleic acid (+99 %)
0.312-0.465 ml	ethanol, absolute
10 mM	Hepes

Preparation:

Increasing amounts of oleic acid were pipetted into different volumes of alcoholic PC-solutions containing 75 micromoles of lipid so as to create a concentration series with a lipid/surfactant ratio beginning with L/S=0.5 and increasing by 0.2 units in each step. Subsequently, each lipid sample was supplemented with 4.5 ml of sterile buffer solution and the mixtures were incubated at 4°C for one day. When the pH value had to be adjusted by addition of 1 M NaOH, the first incubation period was followed by another incubation for 24 hours. In order to obtain a final liposome suspension, each sample was thoroughly mixed and filtered through a polycarbonate filter (0.45 micrometer) into a glass vial which was then kept closed at 4°C.

Characterization:

Permeation resistance is assumed to be proportional to the relative pressure needed to perform a secondary filtration through a 0.2 micrometer filter. In this report this resistance is given in relative units of 1 to 10.

Vesicle size is measured by means of dynamic light scattering at 33 degrees C, using a Malvern Zeta-Sizer instrument. For the analysis of correlation curves, a special variant of the software package "Contin" is employed.

In this experimental series all vesicle sizes are relatively independent of the total concentration of edge active substances, in the range of 300 through 350 nm.

Permeation:

Permeation resistance first increases with decreasing relative concentration of fatty acid in the transfersomes. This trend is not monotonous, however. At a lipid/surfactant-ratio of

Specific data points (0) are shown in figure 1. Vesicles diameters were always measured after permeation experiments.

Composition:

Preparation:

Permeation and Characterization:

Permeation resistance is determined as described in examples

Corresponding measured points are shown as (+) in figure 1.

Composition:

Preparation:

Permeation and Characterization:

The measured permeation resistance corresponds, within the limits of experimental error, to the results given in examples 1-13. Vesicle sizes are also similar in both cases. Immediately after the lipid vesicle have been formulated, their diameters are in the range of 320-340 nm. 8 days later, however, the vesicle size has increased to approx. 440 nm.

Corresponding experimental data is given in figure 2.

Examples 32-39:

Composition:

184.5-199.8 mg	phosphatidylcholine from soy-bean (+95%=PC)
20.5-22.2 mg	phosphatidylglycerol from egg PC (puriss., Na-salt, =PG)
44.9-26.1 µl	oleic acid (+99 %)
0.165-0.178 ml	ethanol, absolute
4.5 ml	Hepes, 10 mM

Preparation:

Anhydrous PG is mixed with an alcoholic solution of PC to give a clear solution with 90 % PC and 10 % PG. Oleic acid is added to this solution; the resulting lipid/surfactant ratios are between 1.6 and 2.8; an isomolar specimen is made in addition to this. All mixtures are suspended in 4.5 ml of a sterile buffer solution to yield a final lipid concentration of 4 % and then left for 3 days, after a pH-value adjustment with NaOH, in order to age.

Permeation and Carrier Characteristics:

For determining the permeation resistance, the same procedure as in examples 1-13 is used. All measured values are, as a rule, smaller than in the case of carriers which contained no charged species but had a similar L/S-ratio. Based on our experiments with a 4 % suspension of PC and oleic acid we conclude that the relatively low total lipid concentration plays only a minor role in this respect.

As in previous examples, a resistance minimum is observed for

the 4 % PC/PG mixtures; this minimum, however, is found with L/S-ratios which are by some 20 % higher than those measured with 8 % lipid suspensions. Vesicle diameters, however, hardly differ from those measured in examples 1-13.

Precise permeation data is shown in figure 3. All quoted diameters were measured immediately after individual permeation experiments. But even 40 days later, they are hardly bigger than at the beginning; figure 4 illustrates this..

Examples 40-49:

Composition:

301.3-335.4 mg	phosphatidylcholine from soy-bean (+95%=PC)
123.3-80.8 μ l	Tween 80 (puriss.)
0.38-0.42 ml	ethanol, absolute
4.5 ml	phosphate buffer, isotonic, sterile

Preparation:

Increasing volumes of Tween 80 are pipetted into appropriate volumes of an alcoholic PC solution. This gives rise to a concentration series with 12.5 through 25 mol-% surfactant (L/S = 4-8). In addition to this, samples with L/S=2 and 3 are also made. After the addition of buffer, lipid vesicles are formed spontaneously: prior to further use, these are made somewhat smaller, with the aid of a 0.8 micrometer filter.

Permeation and Carrier Characteristics:

Permeation resistance is determined in the previously described manner. The corresponding values (0) are shown in the left part of figure 5. As in the case of transfersomes

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which contain oleic acid, a region of anomalously high permeation capability (at $L/S = 6$) can be seen relatively far away from the solubilization point. But it is not before below $L/S=4$ that a maximum permeability is observed. The transfersomal optimum thus is located in a range which differs by a factor of 1.5-2 from the solubilization point.

Precise permeation data is given in figure 5 (wide lines, left panel). The experimental data in right panel documents the vesicle diameters determined after permeability measurements.

Examples 50-61:

Composition:

314.2-335.4 mg	soy-bean phosphatidylcholine (+95 % = PC)
107.2-80.8 μ l	Tween 80 (puriss.)
4.5 ml	phosphate buffer, isotonic, sterile

Preparation:

First Tween 80 and subsequently phosphate buffer are added to appropriate quantities of PC. The resulting mixture is agitated at room temperature for 4 days. The further procedure is as described in examples 40-49.

Permeation and Carrier Characteristics:

Corresponding permeability data is given in figure 5 (thin lines). It confirms, by and large, the results of experiments nos. 40-49.

Examples 62-75:

Composition:

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193-361 mg phosphatidylcholine from soy-bean (grade I,S100)
207.2-38.8 mg Na-cholate, puriss.
4.5 ml phosphate buffer (isotonic with a physiologic
solution)
ethanol, absolute

Preparation:

0.5 ml of a hot solution of S100 in ethanol (2/1, M/V) are mixed with sufficient amounts of bile acid salts which give rise to a concentration series with increasing lipid/surfactant ratio between 1/2 and 5/1. The final total lipid concentration is 8 % in all cases.

Vesicle permeation through constrictions and vesicle solubilization:

The permeation resistance of each sample is measured as in examples 1-13. The vesicle size is determined by means of light scattering. (Radii of particles smaller than 5 nm cannot be measured owing to the insufficient power of the laser source used.)

Corresponding measured data is shown in figure 6. It indicates that the permeation resistance of transfersomes with an L/S ratio below 3.5/1 is very small but that this resistance increases significantly at higher L/S values (left panel); the increase of the mean vesicle diameter above L/S = 2.75 (right panel) is probably a consequence of the decreased flow (and thus of a diminished hydrodynamic shear) caused by the greater permeability resistance in this concentration range.

Within only a few hours after preparation the size of vesicles

just above the solubilization limit (at L/S between 1.25/1 and 2.5/1) is significantly bigger than in the vicinity the 'transfersome optimum'. Such undesired consequences of surfactant activity (cf. Fromherz, P. in: 'Galstone disease, Pathophysiology and Therapeutic Approaches', pp. 27-33, Springer, Berlin, 1990) should always be taken into account. At L/S of approx. 1.25/1, solubilization sets in which leads to the formation of, in our case unmeasurably, small mixed micelles of a size of approximately 5 nm.

Examples 76-91:

Composition:

1.627-0.5442 g phosphatidylcholine from soy-bean (grade I, S100)
4.373-0.468 g Na-cholate, puriss.
60 ml phosphate buffer (physiological)

Preparation:

A 10 % suspension of S100 in phosphate buffer is ultrasonicated at room temperature until the mean vesicle size is approx. 350 nm.

This suspension is divided into three equal volume parts containing 10 %, 1 % and 0.2 % phospholipids. Starting with these preparations, aliquots containing 5 ml of suspension each are prepared. These are supplemented with increasing amounts of sodium cholate (partly in the form of a concentrated micelle suspension), yielding a concentration series with L/S ratios between 1/5 and 5/1. Prior to each permeation- and solubilization measurement, the starting suspension is aged for 1 week at 4°C.

Vesicle permeation through constrictions and vesicle

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Preparation:

A suspension of 1 % desoxycholate containing vesicles is prepared as described in examples 76-91.

Vesicle permeation through constrictions and vesicle solubilization:

The measurements of this experimental series show that vesicles containing desoxycholate are solubilized already at L/S ratios near 1/2, i.e. at an L/S ratio which is by a factor of 2-3 lower than in the case of S100/Na-cholate vesicles.

Examples 99-107:

Composition:

3 mM Suspension of phosphatidylcholine from soy-bean (grade I, S100) in phosphate buffer Na-cholate, puriss.

Preparation:

A 3 mM suspension of S100 in phosphate buffer is partly homogenized at room temperature. 3 ml of this suspension are supplemented each with increasing amounts of sodium cholate in order to create a series with increasing L/S ratios between 1/2 and 12/1. After three days of incubation, these aliquots are ultrasonicated at 55°C, using a 50 % duty-cycle; simultaneously, the optical density at 400 nm of each sample is recorded. An analysis of the resulting experimental data within the framework of a bimodal exponential model reveals two characteristic vesicularization rates (τ_1 and τ_2); these characterize the temporal dependence of the number of lamellae in each vesicle (τ_1) and the changes in the mean size of vesicles (τ_2).

The tau 1 and tau 2 values represented in figure 7 show that the mechanical properties of transfersomes, which are reflected in the value of parameter tau 2, exhibit a similar L/S dependence as the solubilization and permeation tendency (cf. fig. 6). For a 0.2 % suspension investigated in this series 1 cholate molecule per lipid is required for a rapid formation of vesicles (for the formation of largely unilamellar vesicles).

Composition:

A 10 % PC-suspension in isotonic solution of sodium chloride is homogenized at 22°C until the mean size of lipid vesicles is approx. 400 nm. This suspension is then distributed in aliquots of approx. 4.8 ml. A sufficient volume of Triton X-100 is pipetted into each of these aliquots to give a concentration series with nominal PC/Triton ratios in the range of 0.25 through 4 in steps of 0.5. All resulting samples are occasionally mixed and incubated at 4°C for 14 days.

The optical density (OD (400 nm)) of a lipid-triton mixture after a 10-fold dilution provides insight into the vesicle

solubilization; this is represented in the right panel of figure 8. The solubilization limit is approx. 2 triton molecules per PC-molecule. Right below this limit, the optical density (OD (400 nm)) - and thus the vesicle diameters - attain the greatest values. At PC/triton ratios higher than 2,5/1, the change in the optical density of given suspensions is only minimal.

Vesicle permeation and characteristics:

In order to evaluate the permeation capability of the resulting lipid vesicles and transfersomes all suspensions were pressed through fine-pore filters (0.22 micrometer), as described in examples 1-13. The required pressure increases gradually with the decreasing total triton concentration in the suspension; for L/S ratios higher than 2/1 this significantly limits the permeation capability of carriers.

Corresponding results are summarized in the left half of figure 8.

Examples 120-128:

Composition:

403,5-463,1 mg	dipalmitoyl tartaric acid ester, Na-salt
96,5-36,9 mg	laurylsulfate, Na-salt (SDS)
4,5 ml	triethanolamine buffer, pH 7.5

Preparation:

In this test series a synthetic lipid, which is not found in biological systems, was chosen to be the basic transfersome constituent. For each experiment the required dry lipid mass was weighed into a glass vial and mixed with 4.5 ml of buffer.

The latter contained sufficient amounts of sodiumdodecylsulfate (SDS) to give various L/S ratios between 2/1 and 6/1. Well mixed suspensions were first kept at room temperature for 24 hours and subsequently mixed again thoroughly.

Permeation capacity and vesicle characteristics:

Liposomes were pressed through a 0.2 micrometer filter. Simultaneously, the permeation resistance was measured. Vesicles with an L/S ratio below 4/1 can pass the membrane pores very easily; in contrast to this, all vesicles with lower surfactant concentrations or vesicles without edge active components can pass through the porous constrictions only with difficulty (not before an excess pressure of 5 MPa has been created) or not at all (membranes burst).

Examples 129-136:

Composition:

101,6-227 mg	phosphatidylcholine from soy-bean
148,4-22,2 mg	octyl-glucopyranoside (β -octylglucoside),
puriss. 9,85 ml	phosphate buffer, pH 7,3
	ethanol, absolute

Preparation:

Phosphatidylcholine in ethanol (50 %) and octyl-glucopyranoside were mixed in different relative ratios in order to prepare a concentration series with increasing L/S values between 1/4 and 2/1 (and a final total lipid concentration of 2.5 %). Each lipid mixture in a glass vial was then supplemented with 4.5 ml of buffer. Subsequently, the resulting suspension was mixed in an agitator for 48 hours

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at 25°C. The suspension turbidity was greater for the specimen containing lower amounts of octylglucoside. A fine sediment formed in standing samples. Each suspension was mixed thoroughly before the experiment.

Vesicle permeation and characteristics:

All suspensions can be filtered without any problem through filters with a pore diameter of 0.22 micrometer, using only minimal excess pressures of less than 0.1-0.2 MPa; the only two exceptions are the samples with the lowest surfactant concentration. These give rise to small permeation resistances which on the renormalized scale (cf. figures 1-5) corresponds to values of approx. 1 and 2.5, respectively. Figure 9 presents said data.

If the pore diameter is reduced to 0.05 micrometers only suspensions with L/S ratios below 2/1 can still be filtered.

Irrespective of the pore size used all preparations with L/S ratios below 2/1 are unstable; after only a few days, a phase separation is observed between a micelle rich and a vesicle rich phase.

Examples 137-138:

Composition:

43,3 mg, 50 mg	phosphatidylcholine from soy-bean
0.5 mg	phosphatidylethanolamine-N-fluorescein
6,7 mg, 0 mg	cholate, Na-salt, p.a.
5 ml	Hepes-buffer, pH 7,3

Preparation:

Phosphatidylcholine with the addition of 1 %-fluoresceinated lipids with or without desoxycholate is suspended in 5 ml buffer. The lipid/surfactant ratio is 3.5/1 or 1/0. Both 1 %-suspensions are then ultrasonicated in a glass vial for 1.5 or 15 minutes (25 W, 20°C), until the mean vesicle size is approx. 100 nm.

Spontaneous vesicle permeation:

Onto a Millipore-filter with 0.3 micrometer pore diameter, mounted into a Swinney-holder, the lower half of which has been wetted and filled with water, 50 microliters of a lipid suspension are pipetted through the upper opening. By a gentle swinging motion, a relatively homogeneous sample distribution on the filter surface is ensured. After 30 minutes, the holder is carefully opened and left to dry for 60 minutes. Subsequently the water from below the filter is collected and checked fluorimetrically (excitation 490 nm, emission 590 nm). (The determined light intensity is a measure of the permeation capacity.)

The transport of fluorescence markers mediated by surfactants containing transfersomes gives rise to a fluorescence signal of 89.5; in control experiment a value of 44.1 is established. This indicates that transfersomes are capable of transporting encapsulated substances across permeability barriers.

Examples 137-139:

Composition:

43,5, 45,3, 50 mg	phosphatidylcholine from soy-bean
0.5 mg	phosphatidylethanolamine-N-fluorescein
6,5, 4,7, 0 mg	desoxycholate, Na-salt, p.a.

25 ml

Hepes-buffer, pH 7,3

Preparation and results:

Lipid vesicles are made and tested as described in examples 137-138. Measurements show that the transfersomes which contain deoxycholate already show similarly good results at a characteristic L/S ratio of 5/1 as transfersomes which contain cholate at a ratio of L/S=3.5.

Examples 140-142:

Composition:

50 mg;	43,3 mg;	15,9 mg	phosphatidylcholine from soy-bean
0.5 mg			phosphatidylethanolamine-N-fluorescein
0 mg;	6,7 mg;	34,1 mg	cholate, Na-salt, p.a.
5 ml			Hepes-buffer, pH 7,3

Preparation:

Lipid vesicles consisting of phosphatidylcholine and a fluorescent additive were made as in examples 137-138. For this experiment, suspensions with a lipid/surfactant ratio of 1/0, 4/1 and 1/4 were used. The former two contained fluorescent lipid vesicles, the latter a micellar suspension.

Spontaneous penetration into plant leaves:

A fresh onion is carefully opened in order to gain access to individual leaves; these correspond to low-chlorophyll plant leaves. For each measurement, 25 microliters of a fluoresced suspension are applied onto the concave (inner or upper) side of each onion leaf; as a result of this a

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convex droplet with an area of approx. 0.25 square centimeters is formed. (Carriers which contain surfactants can be easily identified owing to their higher wetting capability.) After 90 minutes the (macroscopically) dry lipid film is eliminated with the aid of a water stream from a jet-bottle with a volume of 50 ml.

After this treatment, the 'leaf surface' attains a slightly reddish appearance in the case of surfactant containing transfersomes as well as mixed micelles. Leaves incubated with surfactant-free vesicles cannot be distinguished from the untreated leaves.

Fluorescence measurements using a red filter (excitation through a blue filter from above) show that leaves which were covered with transfersomes are intensively fluorescent throughout the treated area. In certain places extremely brilliant aggregates are detected; these probably correspond to the non-eliminated vesicle-clusters. The fluorescence of leaves which were treated with a surfactant solution in some places is comparably intensive; at other positions their fluorescence is weaker, however, than in the case of transfersome-treated leaves.

The leaves which were treated with standard lipid vesicles do not fluoresce. Over large surface areas they are indistinguishable from the non-treated leaf regions.

This shows that transfersomes can transfer lipophilic substances spontaneously and irreversibly into a plant leaf or its surface. Their penetration capacity exceeds that of preparations containing highly concentrated surfactants, i.e. well established 'membrane fluidizers'.

Examples 143-145:

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Composition:

50 mg; 43,5mg; 17,1 mg phosphatidylcholine from soy-bean
0.5 mg phosphatidylethanolamine-N-fluorescein
0 mg; 4,7 mg; 32,9 mg desoxycholate, Na-salt, p.a.
5 ml Hepes-buffer, pH 7,3

Preparation and results:

The preparation and results are identical with those of experiments 140-142.

Examples 146-148:

Composition:

50 mg; 36,4; 20 mg phosphatidylcholine from soy-bean
0.5 mg phosphatidylethanolamine-N-fluorescein
0 mg; 13,6 mg; 30 mg Brij 35
5 ml Water

Preparation and results:

Preparation and results are comparable to those of experiments 140-142 and 143-145.

Examples 146-150:

Composition:

84,2 to 25 mg phosphatidylcholine from soy-bean 80 %
75 kBq Giberellin A4, 3H-labelled
15,8 to 75 mg polyoxyethylene (23)-laurylether (Brij 35)
1 ml water

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ethanol, absolute

Preparation:

An ethanolic lipid solution (50 %) is mixed with a corresponding amount of an ethanolic solution of giberellin and suspended in 1 ml of water or in appropriate volumes of a surfactant suspension to obtain a total lipid concentration of 10 % and L/S ratios of 8/1, 4/1, 2/1, 1/1 and 1/2. The resulting (mixed) suspension is then briefly homogenized with the aid of ultrasound so that the mean vesicle size is always below 300 nm.

Carrier suspensions are distributed over the surface of 3 leaves of *Ficus Benjaminii*; there, they are permitted to dry for 6 hours. After subsequent intensive washing of each leaf surface with 5 ml of water per square centimetre and destaining with a peroxide solution, the radioactivity in the homogenized plant material is measured scintigraphically in a beta-counter.

Agent transport in plant leaves:

Experiments show, as in examples 140-142, that transfersomes can bring the agent molecules into a leaf surface much more effectively than a micellar solution.

Examples 151-157:

Composition:

32,8-0.64 mg	phosphatidylcholine from soy-bean (purity higher than 95 %, PC)
75 kBq	dipalmitoylphosphatidylcholine tritium- labelled

2,2-34,4 mg bile acid, Na-salt, p.a.
0.32 ml phosphate buffer, pH 7,3

Preparation:

In each case, 35 mg of lipid are mixed with tritium-labelled dipalmitoylphosphatidylcholine in chloroform. After thorough drying under vacuum, the resulting mixture is suspended in 0.32 ml of buffer; the nominal surfactant/lipid ratios are 0; 0.125; 0.167; 0.263; 0.5 and 1 mol/mol. All suspensions are ultrasonicated until they are comparably opalescent, with the exception of the last, optically clear micellar solution. (The time for efficient necessary sonication decreases with increasing S/L). Control measurements with non-radioactive suspensions indicate that the mean 'particle' size in all samples must be around 100 nm. In all experiments approximately 1 day old suspensions are used.

Penetration into and through the intact skin:

On the back of an immobilized nude-mouse anaesthetized with ether six areas of 1x1 cm are marked. Each of these areas is covered with 20 microliters of a carrier suspension at 3x5 minutes intervals. 60 minutes later, the mouse is killed. From each treated area a sample is excised which is then cut to pieces, solubilized and de-stained. The skin-associated radioactivity is measured scintigraphically.

The corresponding results are summarized in figure 10. For comparison, the normalized values are also given which were taken from our patent application pertaining to the use of liposomes for topical anaesthesia. Optimal transfersomes are appreciably better than non-optimal preparations containing surfactants.

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Examples 158-162:

Composition:

31 mg	phosphatidylcholine from soy-bean (purity higher than 95 %, PC)
75 kBq	dipalmitoylphosphatidylcholine tritium- labelled
4 mg	deoxycholate, Na-salt, p.a.
0.32 ml	phosphate buffer, pH 7,3

Preparation:

In each case 35 mg of lipid (PC and deoxycholate) are mixed with tritium-labelled dipalmitoylphosphatidylcholine in a chloroform solution. The resulting lipid mixture is dried and then dissolved in 30 microliters of warm, absolute ethanol. This solution is then mixed with 0.32 ml of a buffer solution (phosphate buffer, 10 mM, 0.9 % NaCl); this corresponds to a lipid/surfactant ratio of 4/1. The resulting suspension is thoroughly mixed and subsequently filtered through filters with pore sizes of 0.8; 0.45; 0.22 and 0.1 micrometers; this gives rise to vesicles with diameters of approx. 800, 400, 200 or 100 nm (suspensions A, B, C, D).

Penetration into and through the skin:

Tails of 2 anaesthetized mice are treated with 50 microlitres of a corresponding vesicle suspension for 15 minutes. Two control animals obtain an i.v. injection of 0.2 ml 1/10 diluted suspension B. After 30, 60, 120, 180, 240 and 360 minutes, blood specimens are drawn from the tail-tip. The radioactivity of these samples, which is determined by means of beta-scintigraphy, is a reliable indication of the systemic concentration of carrier-associated, radioactively labelled

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lipids.

Experimental data show (fig. 11) that systemically applied transfersomes are eliminated from blood comparably as rapidly as standard liposomes. The size of carrier particles appears not to affect the spontaneous penetration into skin. All transfersomes investigated in this study can penetrate intact skin and get into the depth of a body quite effectively within a period of 4 hours at approx. 1 carrier; tendency increasing.

Examples 163-165:

Composition:

88 mg	phosphatidylcholine from soy-bean (purity higher than 95 %, PC)
75 kBq	inulin, tritium labelled
12 mg	deoxycholate, Na-salt, p.a.
100 ml	ethanol, absolute
0.9 ml	isotonic salt solution

Preparation:

100 mg of PC dissolved in 100 ml of warm ethanol, or a corresponding PC/deoxycholate solution ($L/S = 4.5$), are mixed with 0.9 ml of an isotonic salt solution (suspensions A and B, respectively). Each suspension is ultrasonicated until the mean vesicle size is about 150 nm.

12 microlitres of an aqueous solution of tritium-labelled inulin are pipetted into 38 microliters of a freshly prepared suspension of empty liposomes (A) or transfersomes (B). Subsequently, all mixtures are sonicated in closed vials for 60 minutes in an ultrasound bath at room temperature; they are all used for experiments within 24 after vesicle preparation.

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On the abdomen of NMRI-mice in general anaesthesia, which three days before were depilated using medical tweezers, 10 microlitres of a vesicle suspension containing inulin in every case are applied twice at time intervals of approx. 3-5 minutes.

15, 30, 60, 120, 180, 240, 300 and 360 minutes later, 0.05 ml of blood are routinely taken from the tail of a each mouse to be then investigated scintigraphically. 6 hours later the subcutaneous tissues at the application site, as well as liver and spleen of all animals of this experiment are collected. After solubilization and decolouring procedures, these organs are also checked scintigraphically.

The results of this study are collected in figure 12. They show that normal liposomes can hardly mediate a percutaneous inulin uptake; in contrast to this, 6 hours later approx. 1.4 % of this marker which was applied in the form of transfersomes are found in the blood. This transfer sets in approximately 2-3 hours after the application and is not yet completed 6 hours after each application.

After 6 hours in the case of transfersomes, an average of 0.8 % (this corresponds to 24.1 % of the recovered dose) are in the skin at the application site; 0.9 % are found in the liver; spleen contains less than 0.1 % of the absolute dose. In the body (blood, spleen, liver) approximately 73.8 % of the recovered dose are thus found again.

In contrast to this, approximately 2 % of the normal liposomes at the application site can be detected by eye, the corresponding doses in the liver and spleen being below

0.1 %. This corresponds to a recovery of 95.3 % at the application site and 6.7 % of this dose in the body of the test animal.

Example 166:

Composition:

386 mg	phosphatidylcholine from soy-bean (purity > 95 %)
58.5 mg	sodium-cholate (L/S = 3,5)
500 µl	ethanol (96 %)
2.25 ml	0.9 % NaCl solution (per inject.)
2.25 ml	Actrapid HM 40 (corresponds to 90 I.U. of recombinant human insulin)

Preparation:

Samples are prepared essentially as described in examples 62-75. A mixture of aqueous salt solution and human recombinant insulin (with 6.75 mg m-cresole) is mixed with a lipid solution in ethanol. The resulting, opaque suspension is aged over night. 12 hours later, this suspension is pressed through a sterile filter (Anodisc, pore diameter 0.2 micrometers) with the aid of nitrogen gas with excess pressure of 0.25 MPa under sterile conditions to be then filled into the glass container.

The nominal lipid/surfactant ratio is 3.5; the calculated molar surfactant concentration in the lipid double layer is approx. 5/1. This corresponds to 50 % of the concentration required for solubilization.

The mean radius of vesicles in final suspension in this experiment was 97 nm.

0.5 ml of a fresh, insulin containing transference suspension are applied onto the untreated skin of the left forearm of an informed, healthy male volunteer aged 37 years (starved for 18 hours) and distributed over an area of approx. 10 cm². 5 minutes later, additional 300 microlitres of identical suspension are positioned in two halves on the forearm and upper arm, respectively. 5-10 minutes later, the suspension on the upper arm (dose approx. 2,5 mg/cm²) has almost completely disappeared; it has thus nearly completely penetrated into skin. In contrast to this, lipids applied onto the forearm (dose approx. 7.5 mg/cm²) are still well perceptible.

In order to assess the biological activity of insulin, approx. 2 hours before the sample application, a permanent, soft catheter is placed into a vein in the right hand. Every 15-45 minutes, 1-1.5 ml of blood are collected from this catheter; the first 0.5-1 ml thereof are discarded; the remaining 0.5 ml are measured with a standard enzymatic glucose test. In each case three determinations with three to four independent specimens are made. The corresponding experimental data is summarized in figure 13. It shows that transfersomes mediate a significant hypoglycemia in the peripheral blood some 90 minutes after the drug application; this effect lasts for approx. 2 hours and amounts to approx. 50 % of the magnitude of the hypoglycemic effect of a comparable dose of subcutaneously applied insulin; the effect of the former lasts 200 % longer, however.

Examples 167-172:

Composition:

956 mg	phosphatidylcholine from soy-bean (+95 %)
0-26 mg	sodium-deoxycholate
1 mg	prostaglandine E1
1 ml	ethanol absolute
50 ml	0.9 % NaCl solution (per inject.)

Preparation:

1 ml of ethanol is pipetted into a glass flask with 1 mg of prostaglandine. After thorough mixing, the resulting prostaglandine solution is transferred to the appropriate amount of dry lipid in another glass vial. The original flask is flushed once again with the new lipid/prostaglandine solution and subsequently supplemented with 6 ml of an isotonic salt solution. The prostaglandine containing flask is washed twice with 2 ml of 0.9 % NaCl and mixed with the original lipid suspension. The sample is then divided into 5 parts; into individual aliquots sodium-desoxycholate is added at concentrations of 0; 1.6; 3.25; 6.5 or twice 13 mg/ml.

The resulting 10 % suspensions are aged for 24 hours. Subsequently they are either ultrasonicated or filtered manually through a 0.2 micrometer-filter, depending on cholate concentration. The specimens with the highest surfactant concentration are either filtered or ultrasonicated. Finally, the samples are diluted to obtain a final PGE1 concentration of 20 micrograms/ml and kept in dark glass bottles in a refrigerator. Vesicle radius right after sample preparation is 85 nm, two months later 100 nm.

Application and Action:

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In each experiment 0.25 ml of a lipid suspension are applied on neighbouring but not interconnected regions of abdominal skin. 10 minutes later the skin surface is macroscopically dry; 15 minutes later, some of the application sites show a reddish appearance which, according to the test person's statement, is associated with a weak local pain. The intensity of oedema grades as 0, 0, 0, 0-1, 3 and 3 points (on a scale from 1-10).

This shows that merely transfersomes - but not liposomes or sub-optimal surfactant-containing vesicles - can penetrate into intact skin and thereby transfer drugs into body. The precise mode of sample preparation plays no role in this.

Examples 173-175:

Composition:

79.4 mg; 88.5 mg	phosphatidylcholine from soy-bean (+95%)
20.6 mg, 11.5 mg	sodium-deoxycholate
10 µg	hydrocortison
0.1 ml	ethanol absolute
1 ml	phosphate buffer, physiological

Preparation:

Lipids and hydrocortison are mixed as approx. 50 % ethanolic solution and subsequently supplemented with 0.95 ml of phosphate buffer. The resulting, very heterogeneous suspension is treated with ultrasound (25 W, 3-5 min). Specimens with an L/S ratio of 2/1 can be homogenized with ease, specimens with L/S = 4/1 are relatively difficult to homogenize.

Specimens with 1 and 2.5 weight-% result in stable suspensions

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Examples 175-200:

1.1 - 2mg phosphatidylcholine from soy-bean (+95%=PC)
0 - 32.5 mol-% Tween 80
pH 7.2 isotonic phosphate buffer

Different amounts of phospholipid and surfactant in each experiment are weighed or pipetted into 25 ml of buffer at ratios which yield suspensions with 0 -32,5 mol-% of Tween 80 and a constant total lipid concentration of 2 %. Specimens are sterilized by filtering, filled into sterile glass vials and aged for 4 through 34 days. Then, the optical density of each sample is determined. This depends strongly on surfactant concentration but hardly on time within the framework of measuring conditions.

23 specimens each containing 3 ml of an individual lipid suspension are ultrasonicated in closed vials in a bath sonicator. Three, four and six hours later the samples' optical density is determined. Such measurements are repeated with every new sample series after the relative sample positions were exchanged in a systematic manner; the determination of optical density, again, is performed three, four and six hours after the start of sonication. All values corresponding to one concentration are summed up and divided

This procedure is an alternative or a supplement to the permeation resistance measurements as described in examples 40-49. Figure 16 shows, for example, that the amount of surfactant required for good mechanical deformability in the case of Tween 80 is 2-3 times lower than the corresponding solubilization concentration. This result is in good accord with the results of the permeation experiments.

Composition:

Preparation:

Permeation and carrier characteristics:

The already described method for the determination of suspensions permeability resistance is used. Corresponding values are given in the left panel of figure 14 as circles or

crosses (two independent test series). The functional dependence of the samples' permeability resistance as a function of the L/S ratio is similar to that of comparable transfersomes and is illustrated in the right panel of figure 14. The maximum permeation capacity is not reached before the L/S-value is below 3.

Examples 216-235

Composition:

202,0-413 mg phosphatidylcholine from soy-bean (+95%=PC)
298,0-87,0 mg Myrj 49
0.26-0.45 ml ethanol, absolute
4.5 ml phosphate buffer, pH 6,5, 10 mM

Preparation and Characterization:

Transfersomes are made and characterized as described for examples 201-215. Their permeation properties as a function of the relative surfactant concentration in the individual specimen is given in the left panel of figure 15. The right panel gives corresponding equilibrium values; the latter, however, provide no information about vesicle suitability for permeation and agent transport.

Example 236:

Composition:

144,9 mg phosphatidylcholine from soy-bean
24.8 mg desoxycholate, Na-salt
1.45 ml Actrapid HM 100 (145 I.U.)
0.16 ml ethanol, absolute

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Preparation:

Appropriate quantities of both lipids are dissolved in corresponding amounts of ethanol and mixed with a standard solution of insulin. 12 hours later, the crude carrier suspension is homogenized by means of filtration. Average vesicle diameter is 225 ± 61 nm and nominal insulin concentration is 83 I.U. Over an area of appr. 10 square centimeters on the right forearm 0.36 ml (30 I.U.) of insulin in transfersomes are distributed. Blood samples are taken every 10 minutes through a heparinized soft catheter positioned in a vein in the right forearm; the first 0.5 ml are always discarded; the following 0.5-0.8 ml of each sample are sedimented and immediately frozen; the remainder of each sample is used for the determination of blood glucose concentration during the experiment.

Activity:

These liposomes with a relatively high surfactant concentration have only a very limited capability of transporting insulin across skin, as is seen from figure 17. Depending on the choice of data used for evaluation, the lowering of the blood glucose level does not exceed 2 to 5 mg/dl over a period of 30-40 minutes at the most. The effect of a comparable subcutaneous injection is 50 to 200 times higher. Surfactant-containing liposomes, which have not been optimized with regard to their 'transfersomal' properties, are consequently poorly suited for the use as carriers in the case of dermal applications. Surfactant concentration in such carriers thus cannot mediate an optimal agent permeation through skin.

This shows that formulations prepared according to this invention can (still) have a partial activity even if their

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Possible use of transfersomes for the application of antidiabetics, most notably of insulin, which has been discussed above in examples 166 and 236, will be investigated in more detail in the following text.

All these procedures have hardly brought any real improvements for the therapy of diabetes patients - with the exception of jet injection, perhaps; but the latter is only a very refined, technically extremely complicated form of injection and,

Lipids have thus far been discussed as excipients for delayed insulin release in insulin implants (Wang, P.Y Int. J Pharm. 54, 223, 1989); in the form of liposomes they were also suggested for use as vehicles for peroral applications (Patel, 1970), without the therapeutic results really being reproducible, however, (Biochem. Int. 16, 983, 1988). Subsequent publications in the field of insulin containing liposomes, therefore, have dealt with methodological rather than therapeutic issues (Wiessner, J. H. and Hwang, K. J. Biochim. Biophys. Acta 689, 490 1982; Sarrach, D. Stud. Biophys. 100. 95, 1984; Sarrach, D. and Lachmann, U. Pharmazie 40. 642, 1985; Weingarten, C. et al., Int. J. Pharm. 26, 251, 1985; Sammins, M.C. et al., J. Pharm. Sci. 75, 838, 1986; Cervato, G. et al., Chem. Phys. lipids 43, 135, 1987).

It is advantageous to use at least one carrier substance for this purpose from the class of physiologically tolerable polar or non-polar lipids or some other pharmacologically acceptable amphiphiles; well-suited molecules are characterized by their ability to form stable agent carrying aggregates. The preferred aggregate form are lipid vesicles, the most preferred membrane structure is a lipid double layer.

It is, furthermore, considered advantageous if at least one

such substance is a lipid or a lipoid from a biological source or some corresponding synthetic lipid; or else, a modification of such lipids, for example a glyceride, glycerophospholipid, sphingolipid, isoprenoidlipid, steroid, sterine or sterol, a sulfur- or carbohydrate-containing lipid, or any other lipid which forms stable double layers; for example, a half-protonated fluid fatty acid. Lipids from eggs, soy-bean, coconuts, olives, safflower, sunflower, linseed, whale oil, Nachtkerze or primrose oil, etc. can be used, for example, with natural, partly or completely hydrogenated or exchanged chains. Particularly frequently, the corresponding phosphatidylcholines are used; as well as phosphatidyl-ethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidic acids and phosphatidylserines, sphingomyelins or sphingophospholipids, glycosphingolipids (e.g. cerebrosides, ceramidpolyhexosides, sulfateids, sphingoplasmalogenes); gangliosides or other glycolipids are also suitable for the use in transfersomes according to this invention. Amongst the synthetic lipids especially the corresponding dioleoyl-, dilinoleyl-, dilinolenyl-, dilinolenoyl-, diaracidonyl-, dimyristoyl-, less frequently dipalmitoyl-, distearoyl-, phospholipide or the corresponding sphingosin derivatives, glycolipids or other diacyl- or dialkyl-lipids are used; arbitrary combinations of the above-mentioned substances are also useful.

It is advantageous if an edge active substance is a nonionic, a zwitterionic, an anionic or a cationic surfactant. It can also contain an alcohol residue; quite suitable components are long-chain fatty acids or fatty alcohols, alkyl-trimethyl-ammonium-salts, alkylsulfate-salts, cholate-, deoxycholate-, glycodeoxycholate-, taurodeoxycholate-salts, dodecyl-dimethyl-aminoxide, decanoyl- or dodecanoyl-N-methylglucamide (MEGA 10, MEGA 12), N-dodecyl-N,N-dimethylglycine, 3-(hexadecyldimethylammonio)-propanesulfonate, N-hexadecyl-

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sulfobetaine, nonaethyleneglycol-octylphenylether, nonaethylene-dodecylether, octaethyleneglycol-isotridecylether, octaethylene-dodecylether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-cetylstearylether (Cetomacrogol, Cremophor O, Eumulgin, C 1000) polyhydroxyethylene-4-laurylether (Brij 30), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrated castor oil, sorbitane-monolaurate (Arlacel 20, Span 20), especially preferred decanoyl- or dodecanoyl-N-methylglucamide, lauryl- or oleoylsulfate-salts, sodiumdeoxycholate, sodiumglycodeoxycholate, sodiumoleate, sodiumelaidate, sodiumlinoleate, sodiumlaurate, nonaethylene-dodecylether, polyethylene-glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-23-lauryl ether (Brij 35), polyhydroxyethylene-40-stearate (Myrj 52), sorbitane-monolaurate (Arlacel 20, Span 20) etc.

Amongst the most suitable surfactants in these classes of substances are: n-tetradecyl(=myristoyl)-glycero-phosphatidic acid, n-hexadecyl(=palmityl)-glycero-phosphatidic acid, n-octadecyl(=stearyl)-glycero-phosphatidic acid, n-hexadecylene(=palmitoleil)-glycero-phosphatidic acid, n-octadecylene(=oleil)-glycero-phosphatidic acid, n-tetradecyl-glycero-phosphoglycerol, n-hexadecyl-glycero-phosphoglycerol, -n-octadecyl-glycero-phosphoglycerol, n-hexadecylene-glycero-phosphoglycerol, n-octadecylene-glycero-phosphoglycerol, n-tetradecyl-glycero-phosphoserine, n-hexadecyl-glycero-phosphoserine, -n-octadecyl-glycero-phosphoserine, n-hexadecylene-glycero-phosphoserine and n-octadecylene-glycero-phosphoserine.

Total concentration of the basic carrier substance is normally between 0.1 and 30 weight-%; preferably, concentrations between 0.1 and 15 %, most frequently between 5 and 10 % are used.

Total concentration of the edge active substance in the system amounts to 0.1 % through to 99 mol-% of the quantity which is required to solubilize the carrier, depending on each application. Frequently, the optimum is drug dependent - in a concentration range between 1 and 80 mol-%, in particular between 10 and 60 mol-%; most frequently values between 20 and 50 mol-% are favoured.

The concentration of the drug agent in the case of insulin is most frequently in the range between 1 and 500 I.U./ml; concentrations between 20 and 100 I.U./ml are preferred; carrier concentration in the latter case is in the range between 0.1-20 weight-%, frequently between 0.5 and 15 weight-%, most frequently between 2.5 and 10 weight-%.

For preparing a therapeutic formulation, the carrier substances, which are very frequently lipids, are taken as such or dissolved in a physiologically acceptable solvent or a water-miscible solubilizing agent, combined with a polar solution, and made to form carriers.

It is advantageous to use polar solutions containing edge active substances; the latter can also be used with lipids or be contained in a lipid solution.

Carrier formation is preferably initiated by stirring in, by means of evaporation from a reverse phase, by means of an injection or a dialysis procedure, through mechanical agitation, such as shaking, stirring, homogenization,

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ultrasonication, friction, shear, freezing-and-thawing, by means of high-and low-pressure filtration, or any other use of energy.

It may be advantageous to incorporate agents only after carrier formation.

If transfersomes are prepared by means of filtration, materials with a pore size of 0.1-0.8 micrometers, very frequently of 0.15-0.3 micrometers, and particularly preferred of 0.22 micrometers are preferably used; several filters can also be used in combination or in a row.

In the case that transfersomes are made by means of ultrasonication, energy densities in the order of 10-50 kW/litre/minute are preferably used; in stirring or rotary machines 1,000 through to 5,000 revolutions per minute are typically used. If high pressure homogenizers are used, pressures in the order of 300-900 Bar normally ensure sufficient transfersome homogeneity and quality after a single passage; in the latter case even suspensions with 20-30 % lipids can be processed without any difficulty.

It is often sensible to prepare transfersomes only shortly before an application from a concentrate or lyophilisate.

Cryopreservatives, such as oligosaccharides, can facilitate the formation of transfersomes from a lyophilisate.

Standard agent, supporting, or additional substances, in particular the stabilizing, protective, gel-forming, appearance-affecting substances and markers can also be used as described in this application.

The following examples illustrate this invention without

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implying any limits to its general use. Temperatures are given in degree Celsius, carrier sizes in nanometers, and other quantities in common SI units.

Example 237:

Composition:

120 mg	phosphatidylcholine from soy-bean (purity > 95 %)
20 mg	sodium-cholate p.a. (L/D = 3,2)
150 µl	ethanol (96 %)
1.45 ml	Actrapid HM 100 (recombinant human insulin 100 I.U./ml)

Preparation:

This preparation is produced as described in example 166, with only minor modifications. The main difference is that the lipid/insulin mixture is hand-filtered through a 0.22 µm polycarbonate filter (Sartorius) using a 1 ml injection already few minutes after mixture preparation. The final volume of the suspension is 1.2 ml; the nominal lipid/cholate ratio is 2.8/1, in lipid membranes approx. 2.4/1. The final concentration of insulin is approx. 83 I.U./ml; the vesicle radius one day after preparation is 94 nm on the average; one week later, 170 nm.

Application:

One and half hours after the beginning of the experiment, 240 µl of a sterile suspension of insulin containing transfersomes (with 20 I.U.) were taken. These were applied and uniformly smeared at a dose of approx. 0.7 mg lipid/cm² over the inner side of the right forearm of a male test person starved for 18

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hours prior to experiment. 5 minutes later the skin surface is macroscopically dry. Another 45 minutes later no traces of application are visible anymore.

Activity:

At irregular intervals of between 15 and 40 minutes, blood samples are drawn from a soft i.v. catheter placed in the left forearm. The determination of the blood glucose level is performed as described in example 166.

The course in time of the transfersome mediated hypoglycemia is represented in figure 18. The blood glucose level decreases approx. 1.5 hours after drug application by some 10 mg/ml; this artificial hypoglycemia lasts for 4 hours at least and thus attains 70-80 % of the value which can be achieved by a subcutaneous application of a comparable amount of the drug Actrapid. The results of control experiments in which the insulin containing transfersomes are injected subcutaneously are shown as crosses in this figure. The total effect in the latter case is similar to that induced by the free drug injected s.c.

Example 238:

Composition:

216 mg	phosphatidylcholine from soy-bean (487 μ l of a 50 % solution in absolute ethanol)
27 mg	phosphatidylglycerol from egg (98 %)
29.45 mg	oleic acid, puriss.
3 ml	Actrapid HM 100 (recombinant human insulin 100 I.U./ml)
40 μ l	1 N NaOH
20 μ l	1 N NaCl

Preparation:

Lipids are mixed until solution is homogeneously clear. After the addition of an actrapid solution, of alkali and salt solution, an optically opalescent suspension is formed. Filtering of this suspension through a polycarbonate filter with a pore diameter of $0.2 \mu\text{m}$ yields a much less opalescent suspension which consists of vesicles (transfersomes) with a mean diameter of 320 nm.

Application:

Starting glucose concentration in the blood of a test person (70 kg, 37 years, normoglycemic, starved for 24 hours) is measured over a period of 90 minutes for reference. Subsequently, the above-mentioned transfersome suspension with a nominal concentration of 85 I.U. insulin/ml, which has been aged for 12 hours at 4°C , is applied on the right forearm skin (approx. $330 \mu\text{l}$ over an area of approx. 15 cm^2); this corresponds to a total applied dose of 28 I.U.

Activity:

Blood specimens are collected through a heparinized, permanent, soft catheter placed in a vein in the left forearm; 0.5 ml of each sample are sedimented and immediately frozen for further use. The remaining volume is used for the in situ determination of the blood glucose concentration by an enzymatic method. The measured glucose concentration decreases by approx. 8 mg/dl after approx. 2.5 hours and remains diminished for more than 4.4 hours. This corresponds to 75 % of the maximally achievable effect, as concluded from control experiments performed by injecting insulin s.c. The pharmacokinetics of this experimental series is represented in

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figure 19.

Figure 20 gives the results of three typical experiments with insulin. They illustrate the results obtained by one percutaneous and two s.c. drug applications.

Example 239:

Composition:

143 mg	phosphatidylcholine from soy-bean
18 mg	phosphatidylglycerol from egg (98 %)
19.6 mg	oleic acid, puriss.
2 ml	Actrapid HM 100 (200 I.U.)
25 μ l	1 N NaOH

Preparation:

Lipids are weighed into a glass vial and mixed with a standard insulin solution. The resulting opaque suspension is ultrasonicated directly, using a titanium probe-tip (approx. 5 W, 3x5 seconds at 22°C in 60 seconds intervals). The resulting, optically clear but still opalescent suspension contains vesicles with a mean radius of 114 ± 17 nm.

Application and Activity:

The results of this test series are within the limits of experimental error identical to those obtained in example 238.

Example 240:

Composition:

143 mg	phosphatidylcholine from soy-bean
--------	-----------------------------------

18 mg	phosphatidylglycerol from egg (98 %)
20.5 mg	sodium oleate
2 ml	Actrapid HM 100 (200 I.U.)

Preparation:

The lipids are dissolved in a glass vial in 0.15 ml abs. ethanol and then combined with a standard insulin solution. Further procedure is as described in example 239.

Application and Activity:

Over an area of approx. 5 cm² on the forearm skin of a test person a piece of fine-mesh synthetic cloth is fixed. This is then covered with 350 µl of an insulin containing transfersome suspension and left uncovered to dry.

The resulting decrease of the blood glucose level after 4 hours amounts to 7.8 mg/dl and after 6 hours to 8.5 mg/dl. It is thus comparable to the result obtained in experiment no. 238.

Example 241:

The procedure is at first as described in example 238 except that no salt solution is added to the sample suspension; the opaque crude transfersome suspension is divided into two parts. One of these consisting of 50 % of the total volume is passed through a sterile filter; the other half is ultrasonicated for 15 seconds at room temperature at a power of approx. 5 W. The mean diameter of carriers in both halves is similar, 300 nm or 240 nm, respectively.

Example 242:

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The procedure is as described in examples 238 and 240. Transfersomes, however, are filtered one, two and three times in a row. The mean vesicle diameter in the resulting three samples are 300, 240, and 200 nm, resp..

The transfersomes of examples 241 and 242 yield similar hypoglycemic results in biological tests as those of example 238.

Example 243:

Composition:

144,9;152 mg	phosphatidylcholine from soy-bean
24.8;17.6 mg	desoxycholate, Na-salt
1.45;1.55 ml	Actrapid HM 100 (145 I.U.)
0.16 ml	ethanol, absolute

Preparation:

Lipids are weighed into glass vials, dissolved with ethanol and mixed with an insulin solution. The resulting opaque suspension is aged over night and subsequently filtered through a 0.22 micrometer filter at t=12 hours. The nominal insulin concentration is 83 or 84 I.U; the mean vesicle radius in both cases is 112 nm.

Application and Activity:

General experimental conditions are as described in examples 237-239. Transfersome suspensions (0.36 ml, corresponds to 30 I.U.) are applied onto the inner side of a forearm skin in both cases; the blood samples are taken from a soft catheter placed in a vein in the other forearm.

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The results of these two experiments are given in figure 21. They show that preparations with a relatively high surfactant concentration (Sample 1, L/S=3/1) can cause a hardly significant decrease in the blood glucose level; transfersomes close to their optimum, however, with a surfactant concentration lower by approx. 30 % (L/S=4.5/1), cause a very pronounced 'hypoglycemia' which lasts for many hours.

This is another proof that the transfersomes tend to transport drugs through intact skin according to a completely new principle of action which is dissimilar to that of classical pharmaceutical formulations.

This example, in addition to example 236, furthermore, suggests the following conclusion: for the systems investigated, also surfactant concentrations can be used which are remote from the transfersomal optimum (without the carrier activity being lost completely); notwithstanding this, particularly advantageous results are obtained when the surfactant concentration has been determined and chosen to be in a range which ensures maximum carrier elasticity and thus permeation capability of the transfersomes in combination with sufficiently high carrier stability to dissolution, bursting, agent loss, etc.

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Claims:

1. Preparation for the application of agents in the form of minute droplets of fluid, in particular with a membrane-like coating consisting of one or several layers of amphiphilic molecules or of one amphiphilic carrier substance, for transporting agents into and through natural barriers and constrictions such as skin and similar materials, characterized by the fact that each preparation contains an edge active substance at a concentration which amounts up to 99 mol-% of the concentration of this substance required to solubilize the droplet.
2. Preparation according to claim 1, wherein the concentration of edge active substance amounts to at least 0.1 mol-%, in particular between 1 and 80 mol-%, preferably between 10 and 60 mol-%, and particularly preferred between 20 and 50 mol-% of the solubilization-inducing concentration of edge active substances, whereby the edge activity of a droplet unit is preferably close to approx. 10 Piconewton or less.
3. Preparation according to claims 1 or 2, characterized by the fact that the preparation contains an amount of an amphiphilic substance as a carrier or as a basis for the membrane-like envelope of the droplet forming hydrophilic fluid, the agent being contained in the carrier substance, in the shell, and/or in the droplet material itself.
4. Preparation as claimed in claim 3, wherein said amphiphilic substance is a lipid-like material and said edge active substance is preferably a surfactant.

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5. Preparation as claimed in one of claims 1 through 4, wherein the content of said amphiphilic substance for the applications on human or animal skin amounts to 0.01 through 30 weight-% of the preparation mass, preferably between 0.1 and 15 weight-% and particularly preferred between 5 and 10 weight-%.
6. Preparation as claimed in one of claims 1 through 4, wherein the content of the amphiphilic substance in the formulation for application on plants is 0.000001 through 10 weight-%, preferably between 0.001 and 1 weight-% and particularly preferred between 0.01 and 0.1 weight-%.
7. Preparation as claimed in any one of the preceding claims, wherein an agent is an adrenocorticostatic, a β -adrenolytic, an androgen or antiandrogen, antiparasitic, anabolic, anaesthetic or analgesic, analeptic, antiallergic, antiarrhythmic, antiarterosclerotic, antiasthmatic and/or bronchospasmolytic, antibiotic, antidrepressant and/or antipsychotic, antidiabetic, an antidote, antiemetic, antiepileptic, antifibrinolytic, anticonvulsive, an anticholinergic, an enzyme, coenzyme or a corresponding inhibitor, an antihistaminic, antihypertonic, a biological inhibitor of drug activity, an antihypotonic, anticoagulant, antimycotic, antimyasthenic, an agent against Morbus Parkinson, an antiphlogistic, antipyretic, antirheumatic, antiseptic, a respiratory analeptic or a respiratory stimulant, a broncholytic, cardiotonic, chemotherapeutic, a coronary dilatator, a cytostatic, a diuretic, a ganglium-blocker, a glucocorticoid, an antiflow agent, a haemostatic, hypnotic, an immunoglobuline or its fragment or any other immunologically active substance, a bioactive carbohydrate (derivative), a contraceptive, an anti-migraine agent, a mineralcorticoid, a morphine-

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antagonist, a muscle relaxant, a narcotic, a neuraltherapeutic, a nucleotide, a neuroleptic, a neurotransmitter or some of its antagonists, a peptide(derivative), an ophthalmic, (para)-sympaticomimetic or (para)sympathicolytic, a protein(derivative), a psoriasis/neurodermitis drug, a mydriatic, a psychostimulant, rhinologic, any sleep-inducing agent or its antagonist, a sedating agent, a spasmolytic, tuberlostatic, urologic, a vasoconstrictor or vasodilatator, a virustatic or any wound-healing substance, or several such agents.

8. Preparation as claimed in one of claims 1 through 6, wherein said agent is a growth modulating substance for living organisms.
9. Preparation as claimed in one of claims 1 through 6, wherein said agent exerts some biocidal activity and particularly is an insecticide, a pesticide, a herbicide or a fungicide.
10. Preparation as claimed in one of claims 1 through 6, wherein an agent is an attractant, in particular from the class of pheromones.
11. A method for manufacturing preparations for the application of agents in the form of minute droplets of a fluid, in particular in a membrane-like 'envelope' consisting of one or several layers of amphiphilic molecules, or supplemented with an amphiphilic carrier substance, in particular for the transport of agents in and through natural barriers and constrictions, such as skin and the like, characterized by the fact that the concentration of an edge active substance required for the solubilization of a carrier entity is determined and

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then an amount of the edge active substance which is close to the former concentration but still guarantees a sufficient carrier stability and permeation capability is used for the preparation.

12. Method as claimed in claim 11, wherein the stability and the permeation capacity of the fluid 'droplet' are determined by means of filtration, if required under pressure, through a fine-pore filter or by means of any other controlled mechanical fragmentation.
13. Method as claimed in claims 11 or 12, wherein the content of said edge active substance is between 0.1 and 99 mol-%, and in particular between 1 and 80 mol-%, preferably between 10 and 60 mol-% and most preferred between 20 and 50 mol-% of the concentration at which solubilization of the carrier is achieved.
14. Method as claimed in one of claims 11 through 13, wherein said mixture of substances required for the formation of a preparation is subjected to filtration, ultrasonication, stirring, agitating or any other mechanical fragmentation.
15. Preparation as claimed in one of claims 1 through 10, wherein said preparation for non-invasive application contains at least one antidiabetic agent, in particular insulin.
16. Preparation as claimed in claim 15, characterized by the fact that it contains a physiologically compatible polar or non-polar lipid as an amphiphilic carrier substance, the carrier membrane preferably having a double layer structure.

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17. Preparation as claimed in claim 16, wherein the amphiphilic substance is a lipid or a lipoid from any biological source or a corresponding synthetic lipid, or else comprises a modification of such lipids, a glyceride, in particular glycerophospholipid, isoprenoidlipid, sphingolipid, steroid, sterin or sterol, a sulfur- or carbohydrate-containing lipid, or else any other lipid which forms stable double layers, preferably a half-protonated fluid fatty acid, and preferably a phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, a phosphatidic acid, a phosphatidylserin, a sphingomyelin or sphingophospholipid, glycosphingolipid (e.g. cerebroside, ceramidepolyhexoside, sulfatide, sphingoplasmalogene), a ganglioside or any other glycolipid or a synthetic lipid, preferably a dioleoyl-, dilinoleyl-, dilinolenyl-, dilinolenoyl-, diarachidoyl-, dimyristoyl-, dipalmitoyl, distearoyl, phospholipid or corresponding sphingosinderivative, a glycolipid or any other diacyl- or dialkyl-lipid.
18. Preparation as claimed in one of claims 15 through 17, containing several edge active substances.
19. Preparation as claimed in one of claims 15 through 18, wherein said edge active substance is a nonionic, a zwitterionic, an anionic or a cationic surfactant, in particular a long-chain fatty acid or a long-chain fatty alcohol, an alkyl-trimethyl-ammonium-salt, alkylsulfate-salt, cholate-, deoxycholate-, glycodeoxycholate-, taurodeoxycholate-salt, dodecyl- dimethyl-aminoxide, decanoyl- or dodecanoyl-N- methylglucamide (MEGA 10, MEGA 12), N-dodecyl-N,N- dimethylglycine, 3-

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(hexadecyldimethylammonio)-propane-sulfonate, N-hexadecyl-sulfobetaine, nonaethylene-glycol-octylphenylether, nonaethylene-dodecylether, octaethyleneglycol-isotridecylether, octaethylene-dodecylether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-cetylstearyl ether (Cetomacrogol, Cremophor O, Eumulgin, C 1000) polyhydroxyethylene-4-laurylether (Brij 30), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrated castor oil, sorbitane-monolaurate (Arlacel 20, Span 20), particularly preferred decanoyl- or dodecanoyl-N-methylglucamide, lauryl- or oleoylsulfate-salts, sodiumdeoxycholate, sodiumglycodeoxycholate, sodiumoleate, sodiumelaidate, sodiumlinoleate, sodiumlaurate, nonaethylene-dodecylether, polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-40-stearate (Myrj 52) and/or sorbitane-monolaurate (Arlacel 20, Span 20) and lysophospholipid, such as n-octadecylen(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-dilauryl-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine and corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipids.

20. Preparation as claimed in one of claims 15 through 19, characterized by the fact that it contains 1 through 500 I.U. insulin/ml as agent, preferably between 20 and 100

I.U. insulin/ml and the concentration of the carrier substance in the preparation is in the range of 0.1 through 20 weight-%, in particular between 0.5 and 15 weight-%, particularly preferred between 2.5 and 10 weight-%.

21. Preparation as claimed in one of claims 15 through 20, characterized by the fact that a phosphatidylcholine and/or a phosphatidylglycol is used as an amphiphilic substance, and that a lysophosphatidic acid or lysophosphoglycerol, a deoxycholate-, glycodeoxycholate- or cholate salt, a laurate, myristate, oleate, palmitoleate, or a corresponding phosphate- or sulfate-salt, and/or a Tween- or a Myrj-surfactant is used as an edge active substance, recombinant human insulin being the preferred agent.
22. Preparation as claimed in one of claims 15 through 21, wherein the radius of said vesicular droplets in a preparation is between approx. 50 and approx. 200 nm, preferably between approx. 100 and 180 nm.
23. A method for the preparation of a formulation for the non-invasive application of antidiabetic agents, wherein said liposome-like droplets are formed from at least one amphiphilic substance, at least one hydrophilic fluid, at least one edge active substance, and at least one antidiabetic agent which together form the preparation.
24. Method as claimed in claim 23, wherein the edge active substance and the amphiphilic substance, and the hydrophilic substance and the agent are separately mixed together and, if required, dissolved in a solution, the resulting mixtures or solutions then being combined

as one mixture to induce the formation of carrier particles, particularly by action of mechanical energy.

25. Method as claimed in claims 23 or 24, wherein said ampiphilic substance is either used as such or dissolved in a physiologically compatible solvent which is very frequently miscible with hydrophilic fluids, in particular water, or in a solvation mediating agent together with a polar solution.
26. Method as claimed in claim 25, wherein the polar solution contains at least one edge active substance.
27. Method as claimed in one of claims 23 through 26, characterized by the fact that the formation of droplets is induced by substance addition into a fluid phase, evaporation from a reverse phase, using an injection- or dialysis procedure, with the aid of mechanical stress such as shaking, stirring, homogenizing, ultrasonication, shear, freezing and thawing, or high- or low-pressure filtration.
28. Method as claimed in claim 27, characterized by the fact that the formation of droplets is caused by filtration the filtering material having pore diameters of 0.1 through 0.8 μm , in particular with 0.15 through 0.3 μm , especially preferred 0.22 μm , several filters being sometimes used in a sequence.
29. Method as claimed in one of claims 23 through 28, wherein inclusion of said agents occurs at least partly after the droplet formation.
30. Method as claimed in one of claims 23 through 29, wherein liposome-like droplets are prepared just before

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their application from a suitable concentrate or a lyophylisate.

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Abstract

The invention relates to a preparation for the application of agents in the form of minuscule droplets of fluid, in particular provided with membrane-like structures consisting of one or several layers of amphiphilic molecules, or an amphiphilic carrier substance, in particular for transporting the agent into and through natural barriers such as skin and similar materials. The preparation contains a concentration of edge active substances which amounts to up to 99 mol-% of the agent concentration which is required for the induction of droplet solubilization. Such preparations are suitable, for example, for the non-invasive applications of antidiabetics, in particular of insulin. The invention, moreover, relates to the methods for the preparation of such formulations.

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FIG. 1

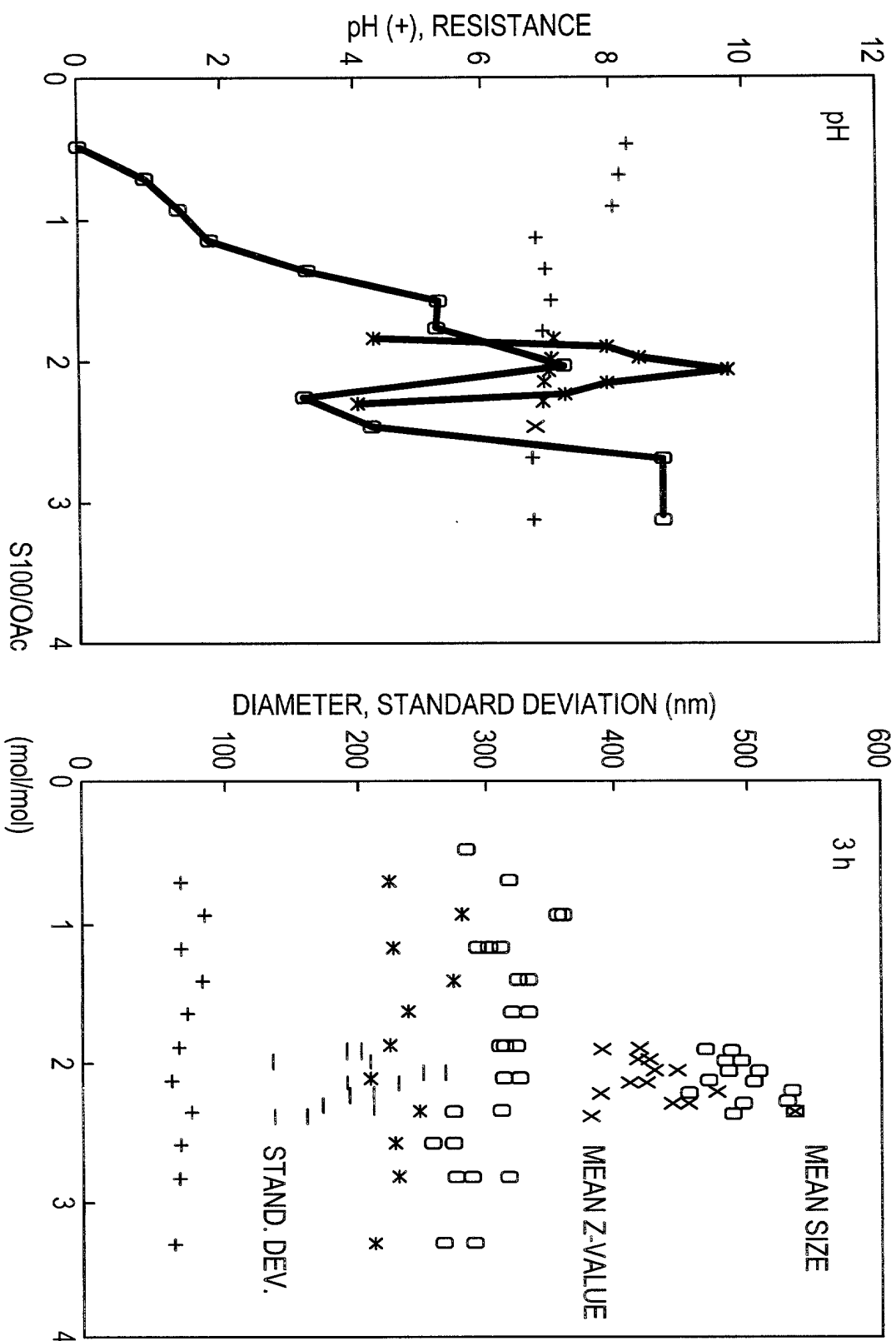


FIG. 2

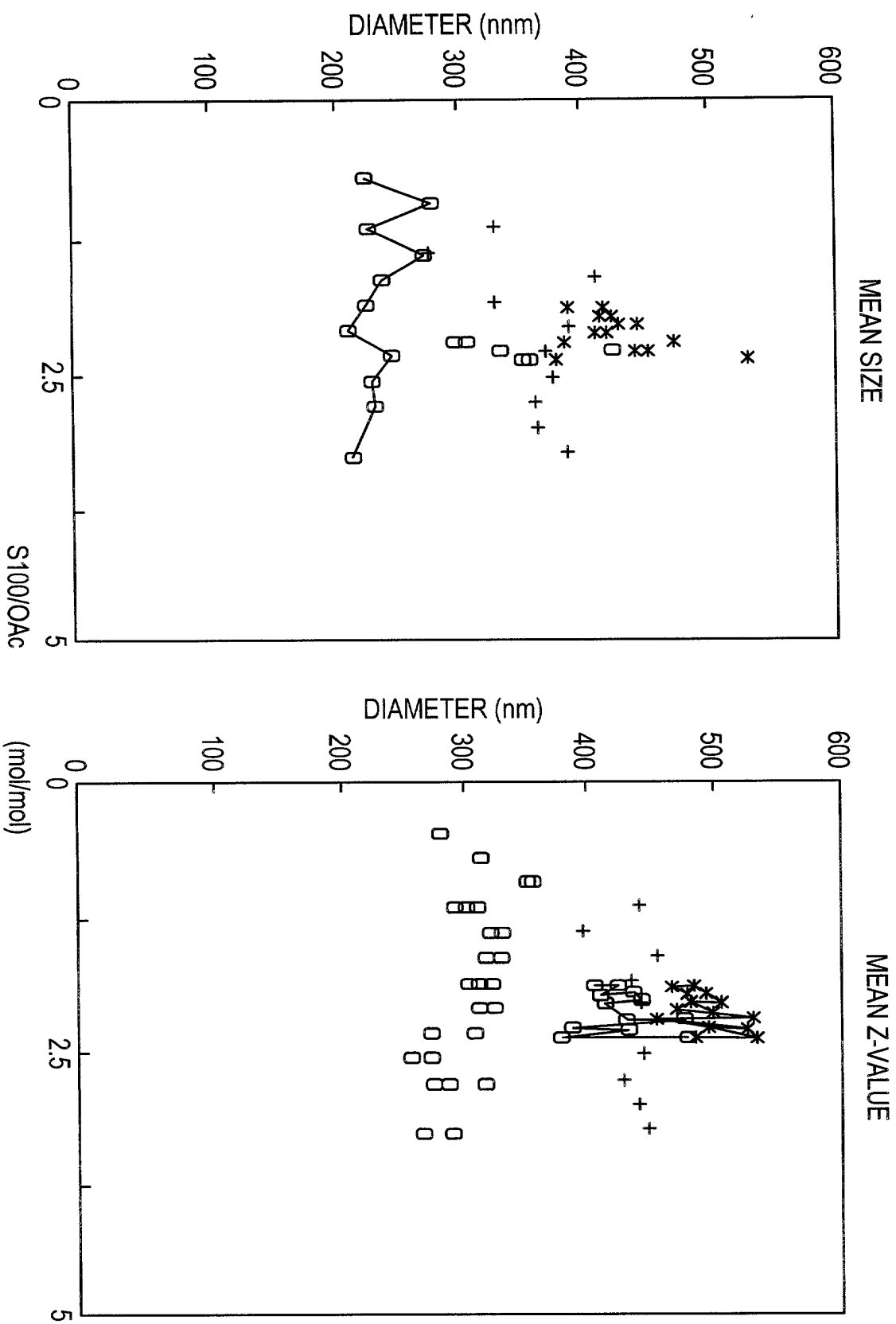


FIG. 3

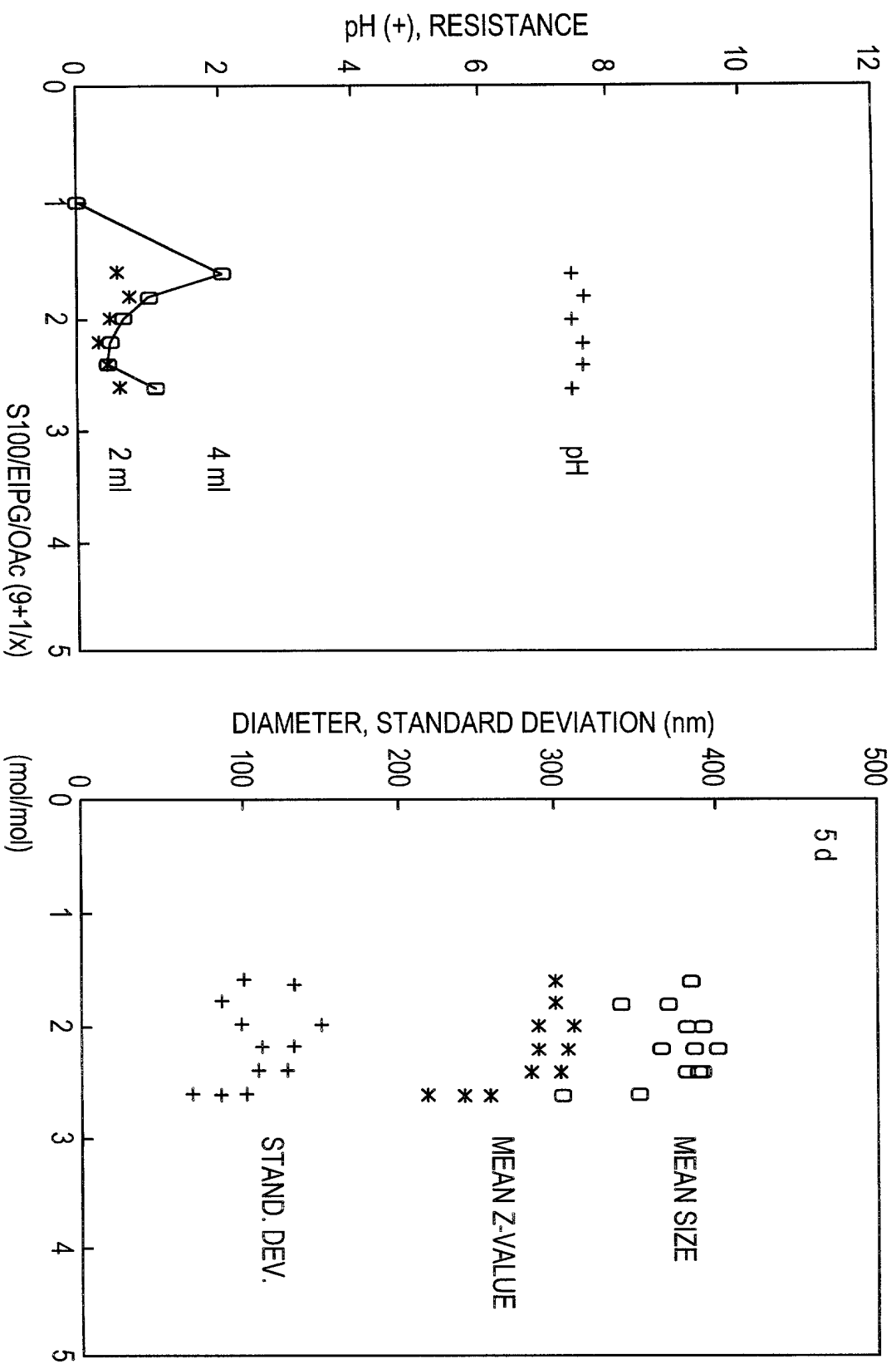


FIG. 4

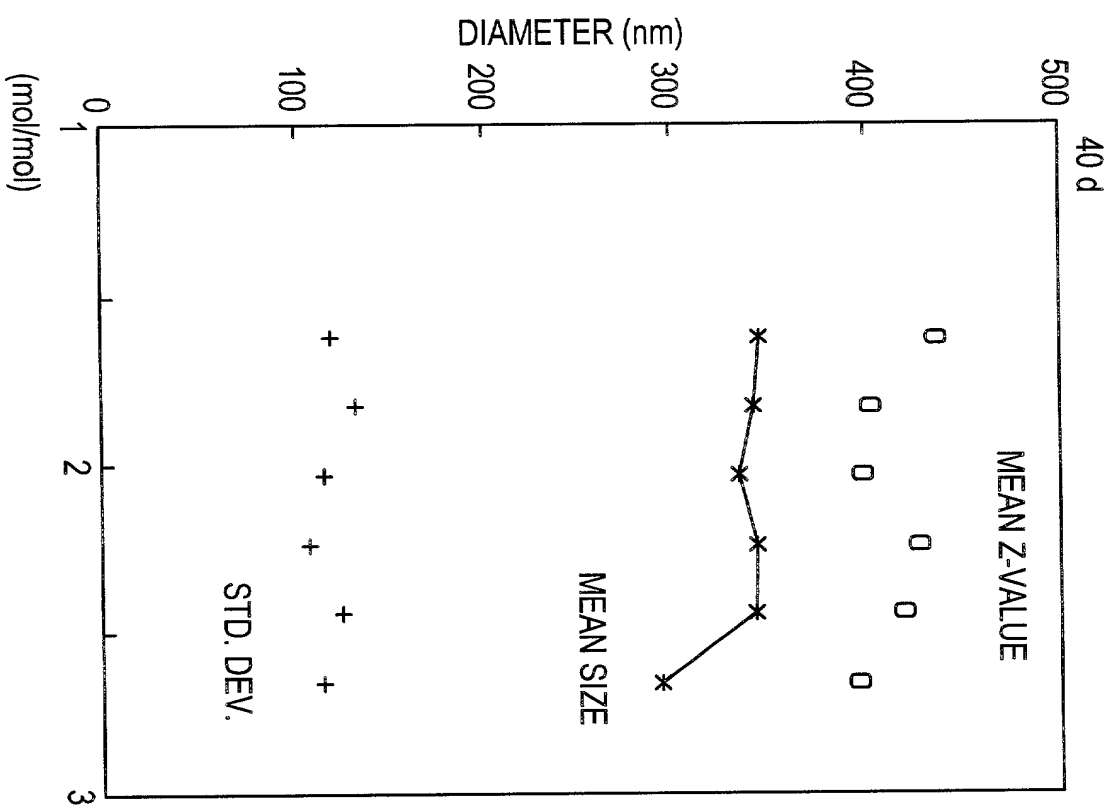
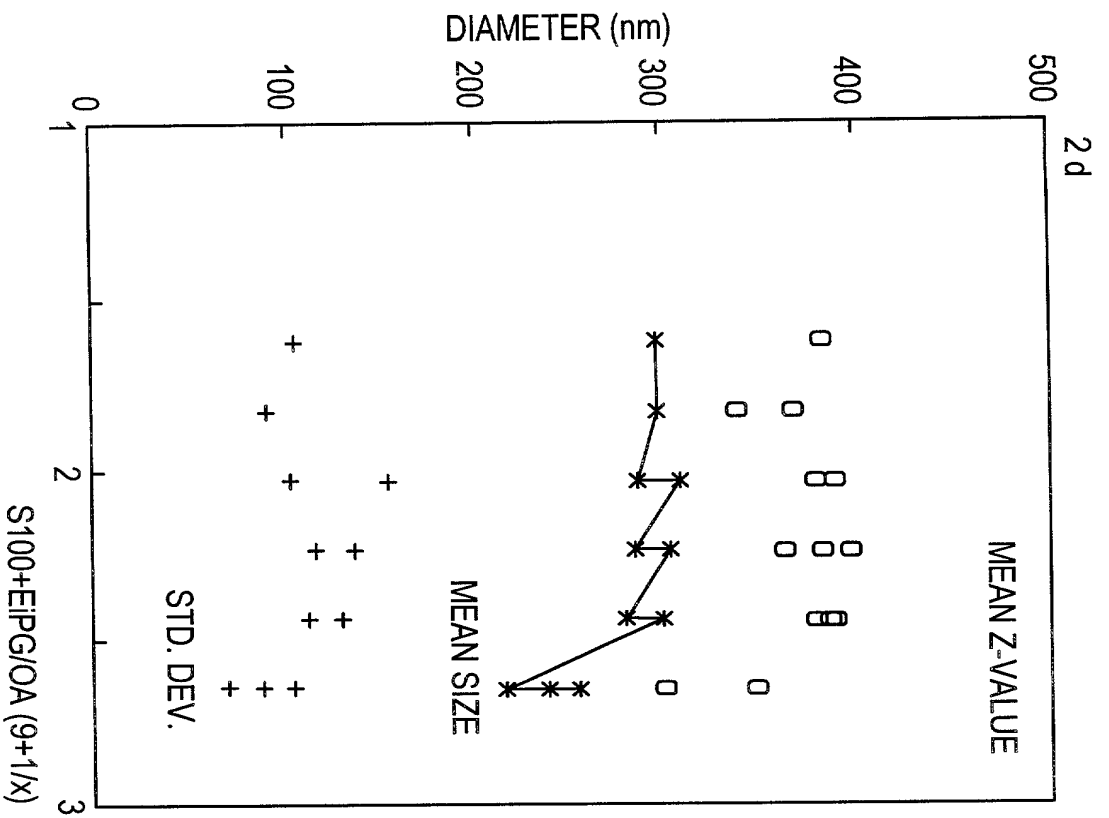


FIG. 5

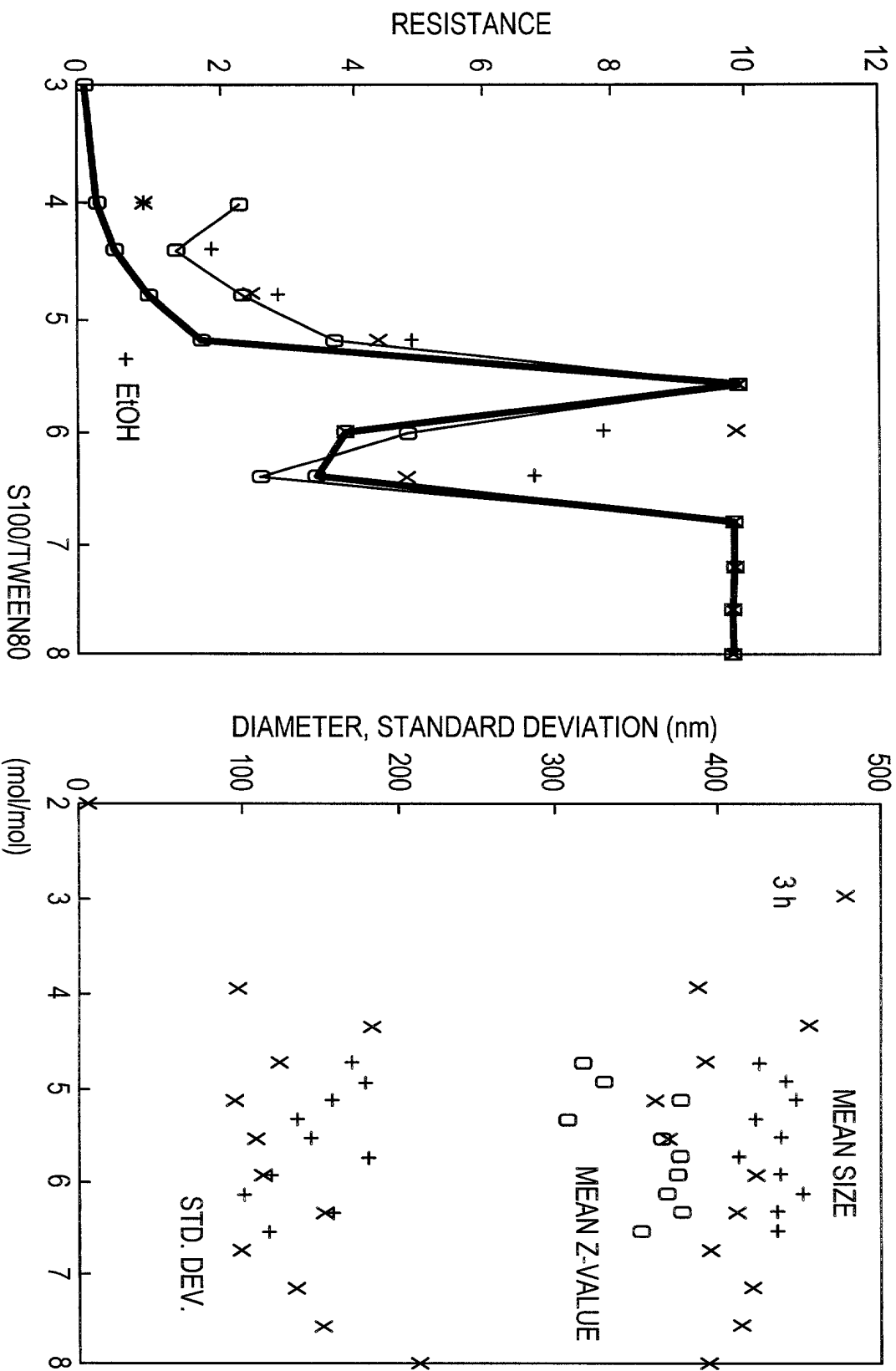
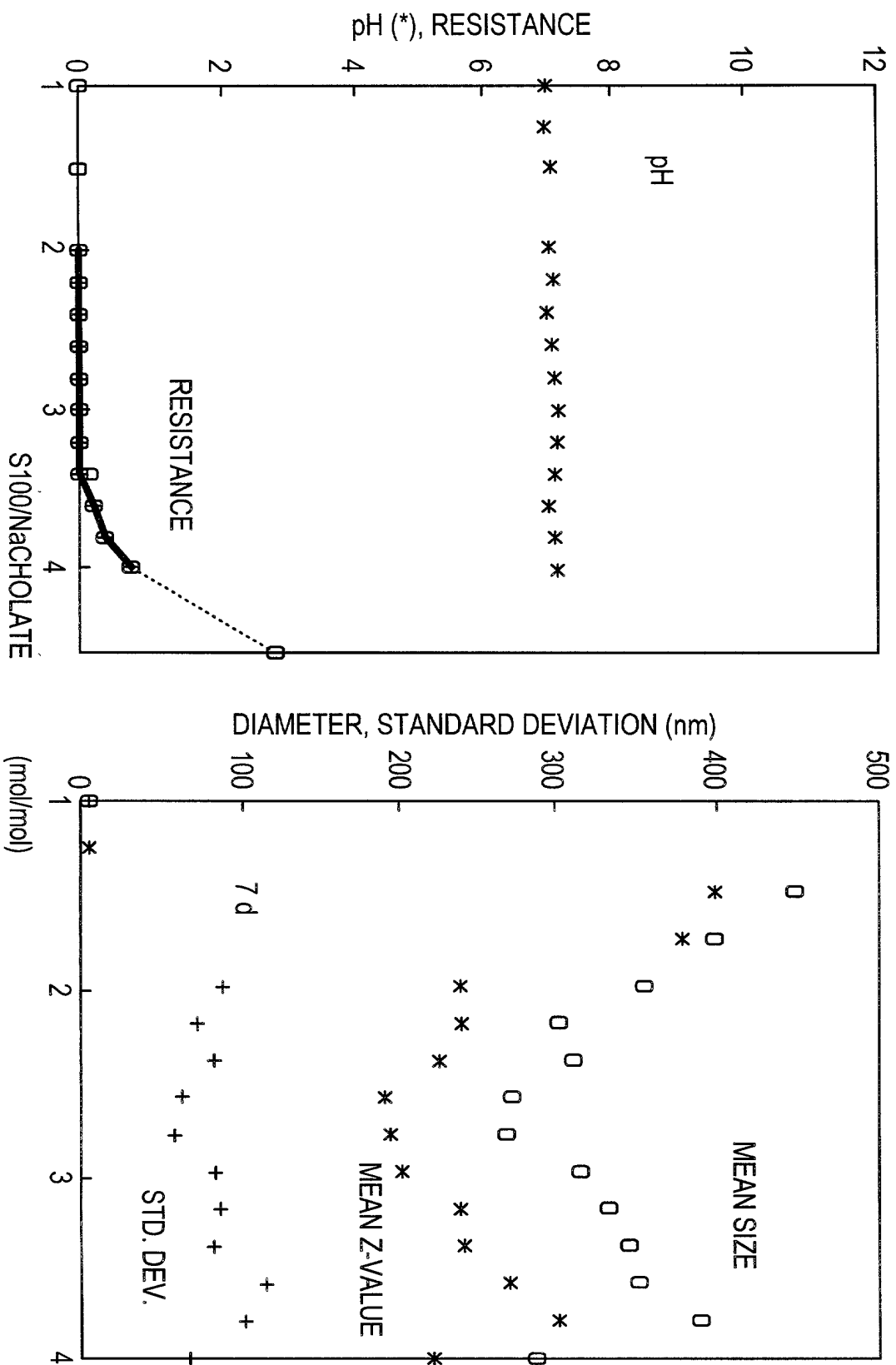


FIG. 6



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FIG. 7

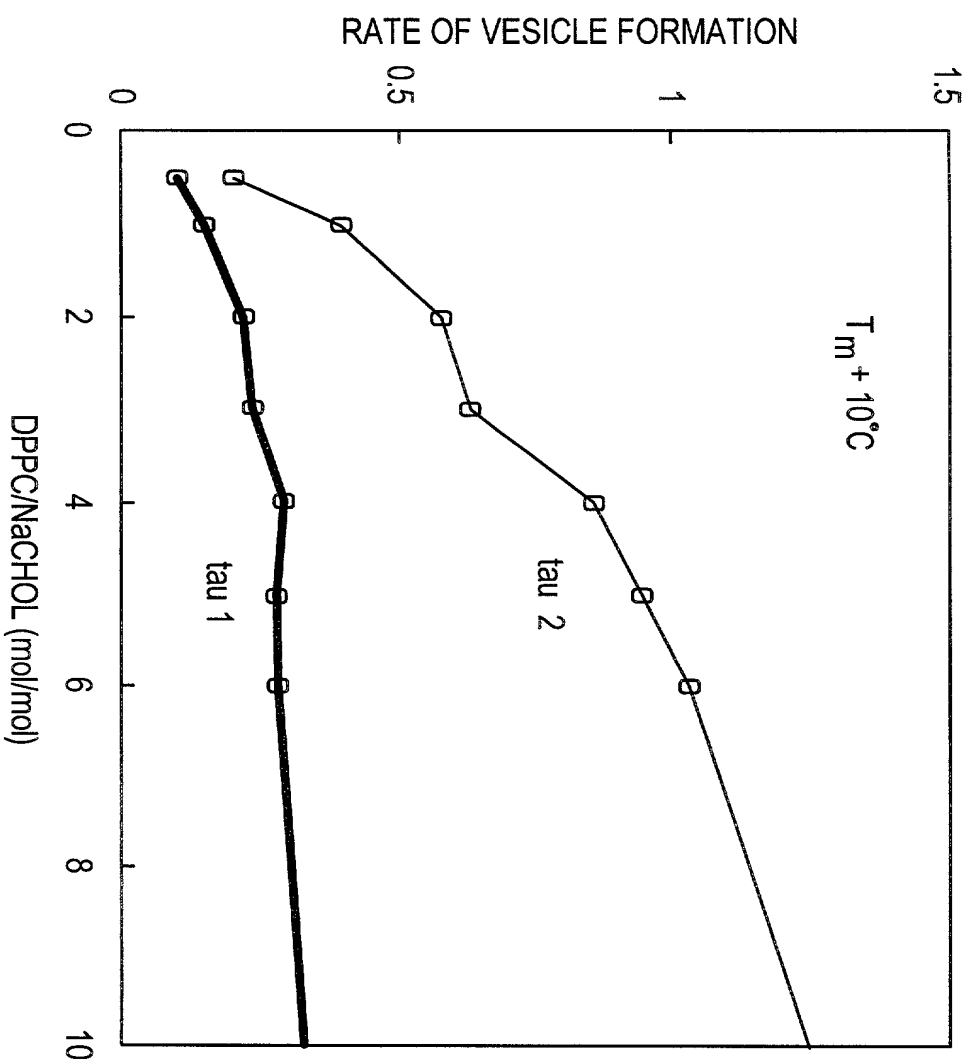


FIG. 8

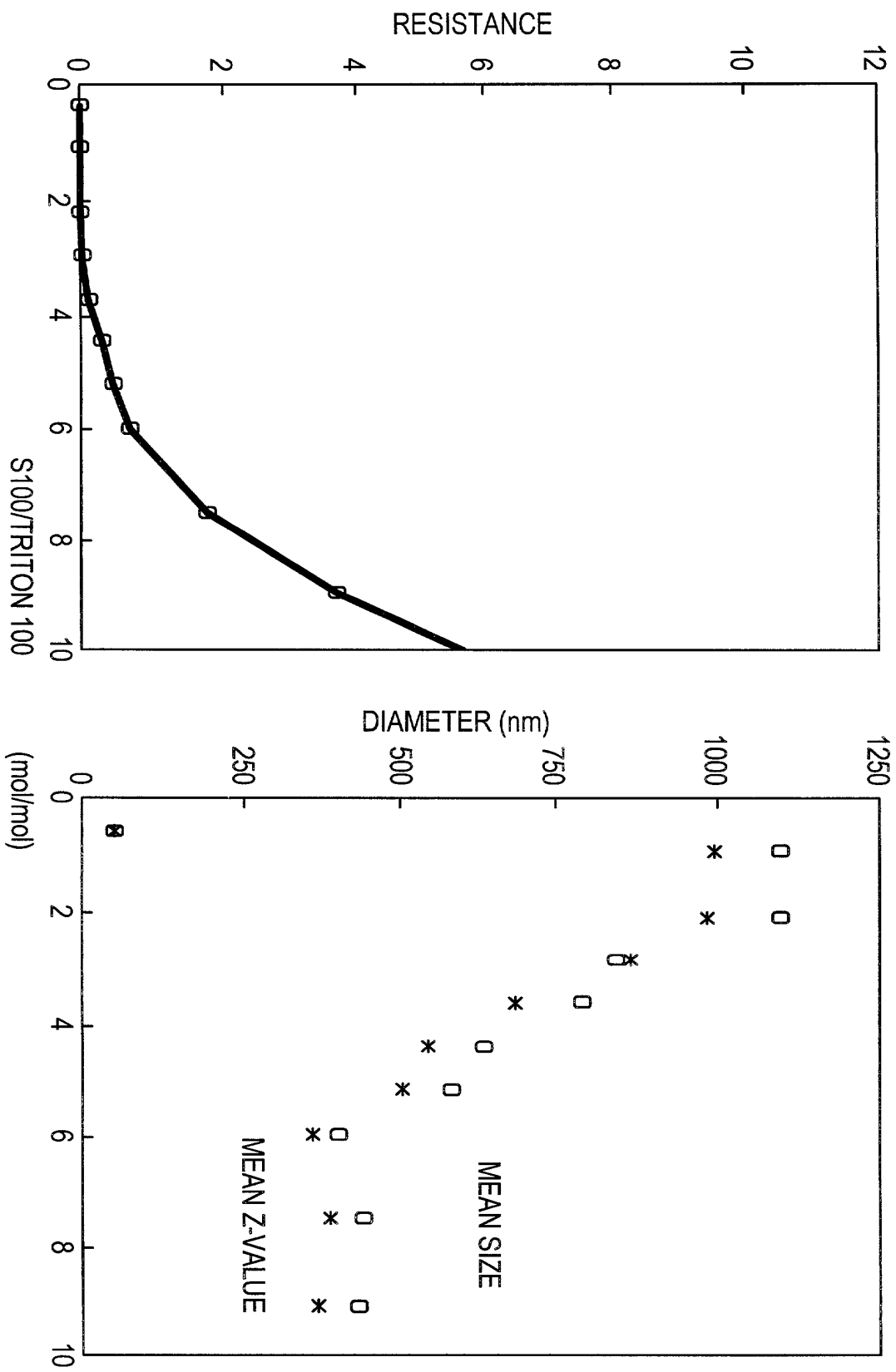


FIG. 9

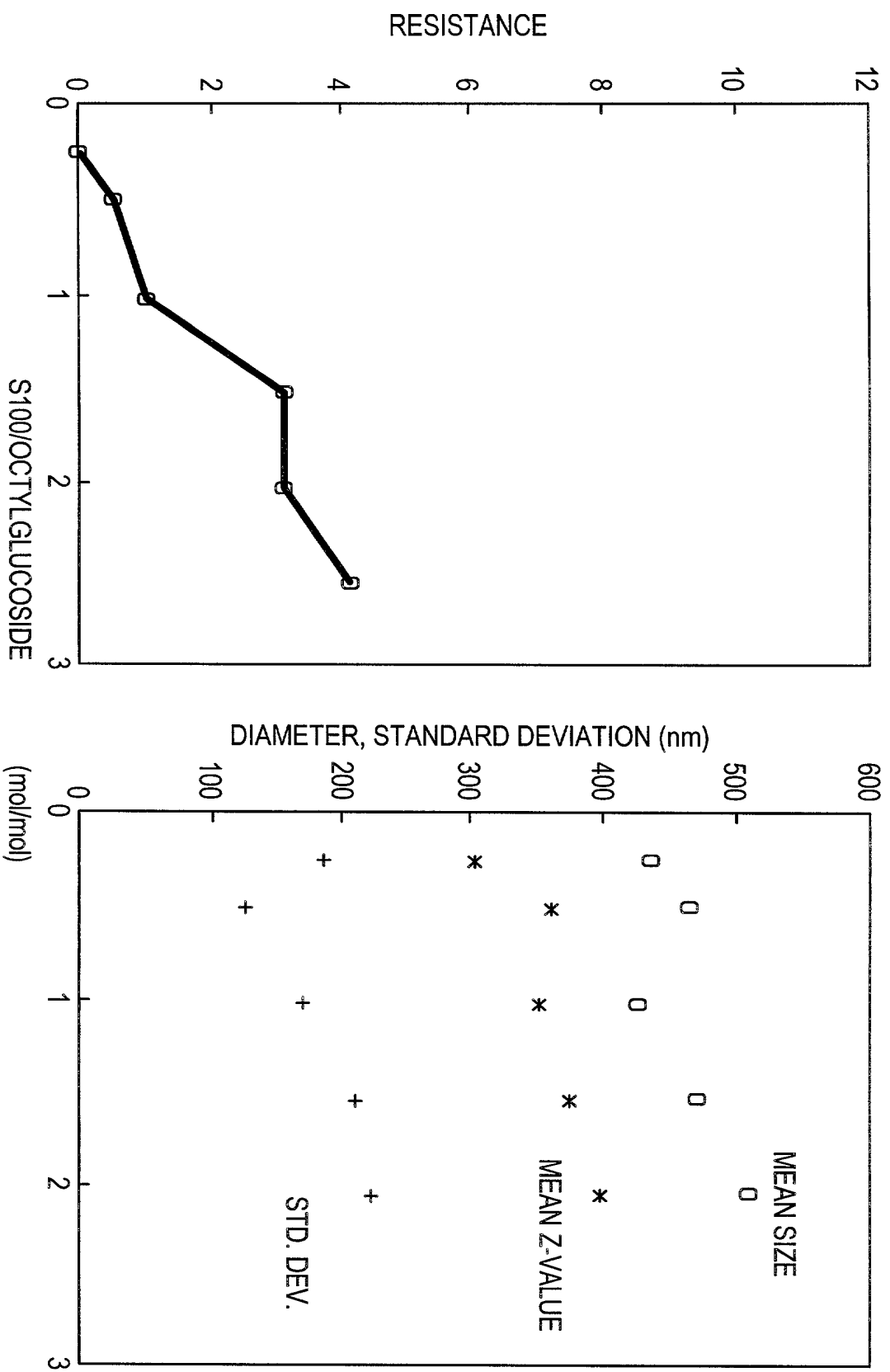
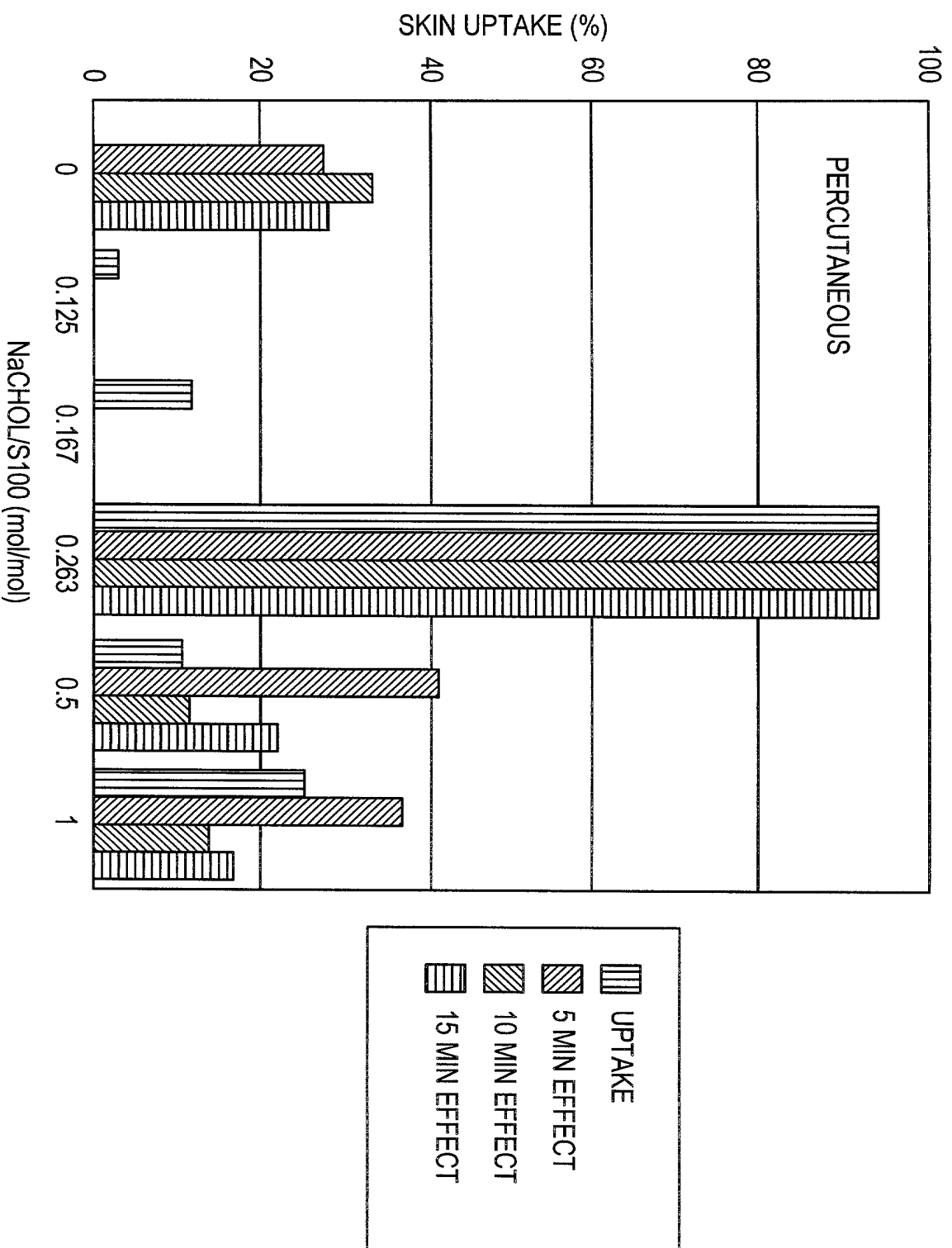


FIG. 10



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FIG. 11

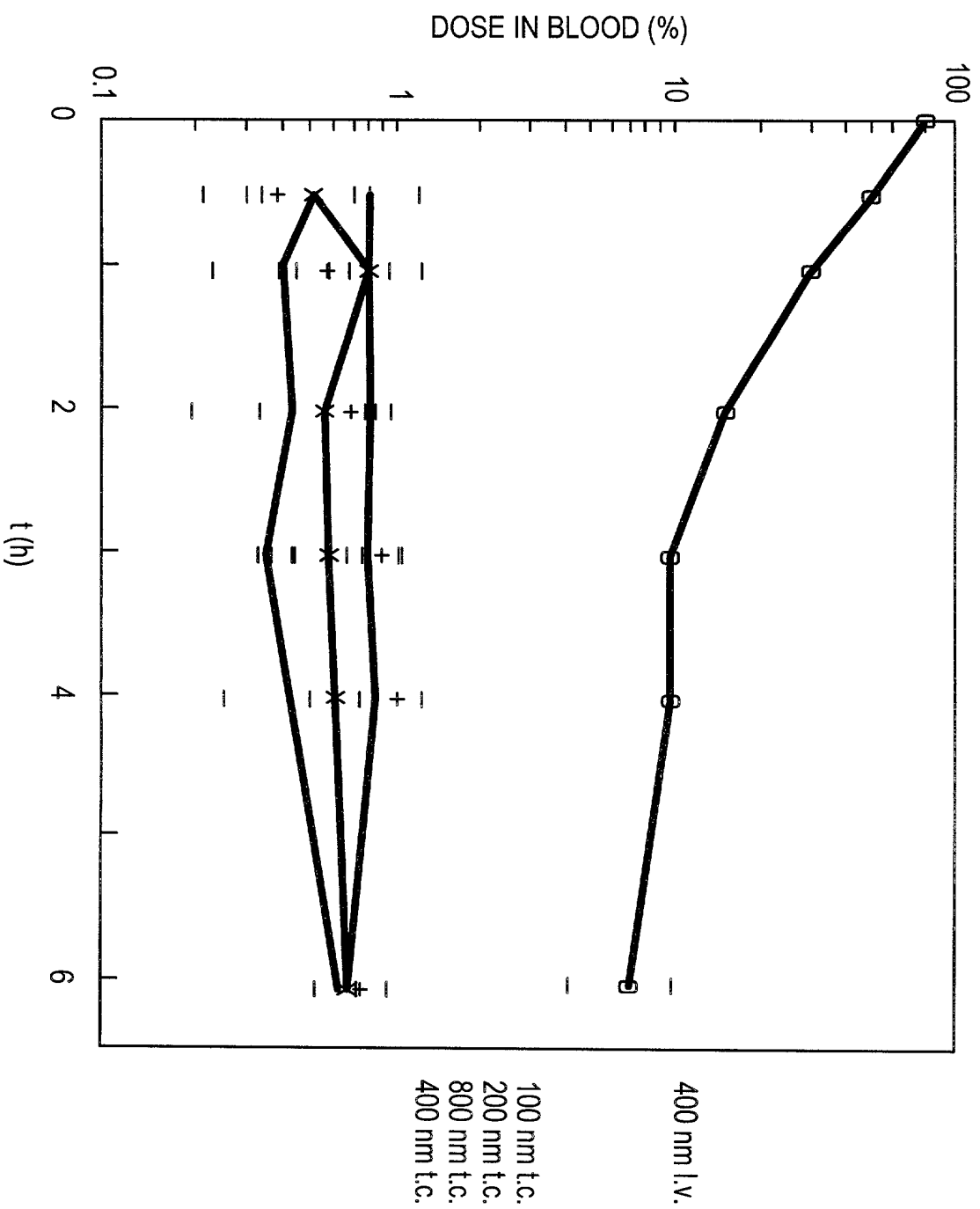


FIG. 12

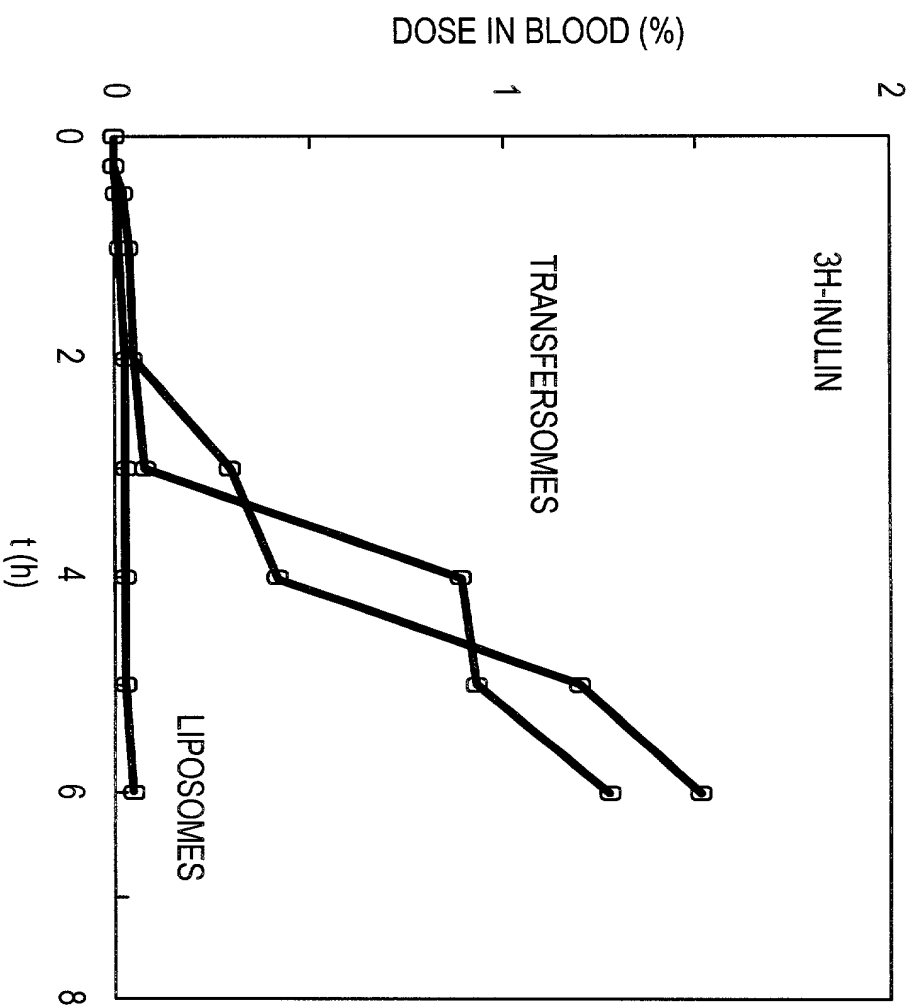


FIG. 13

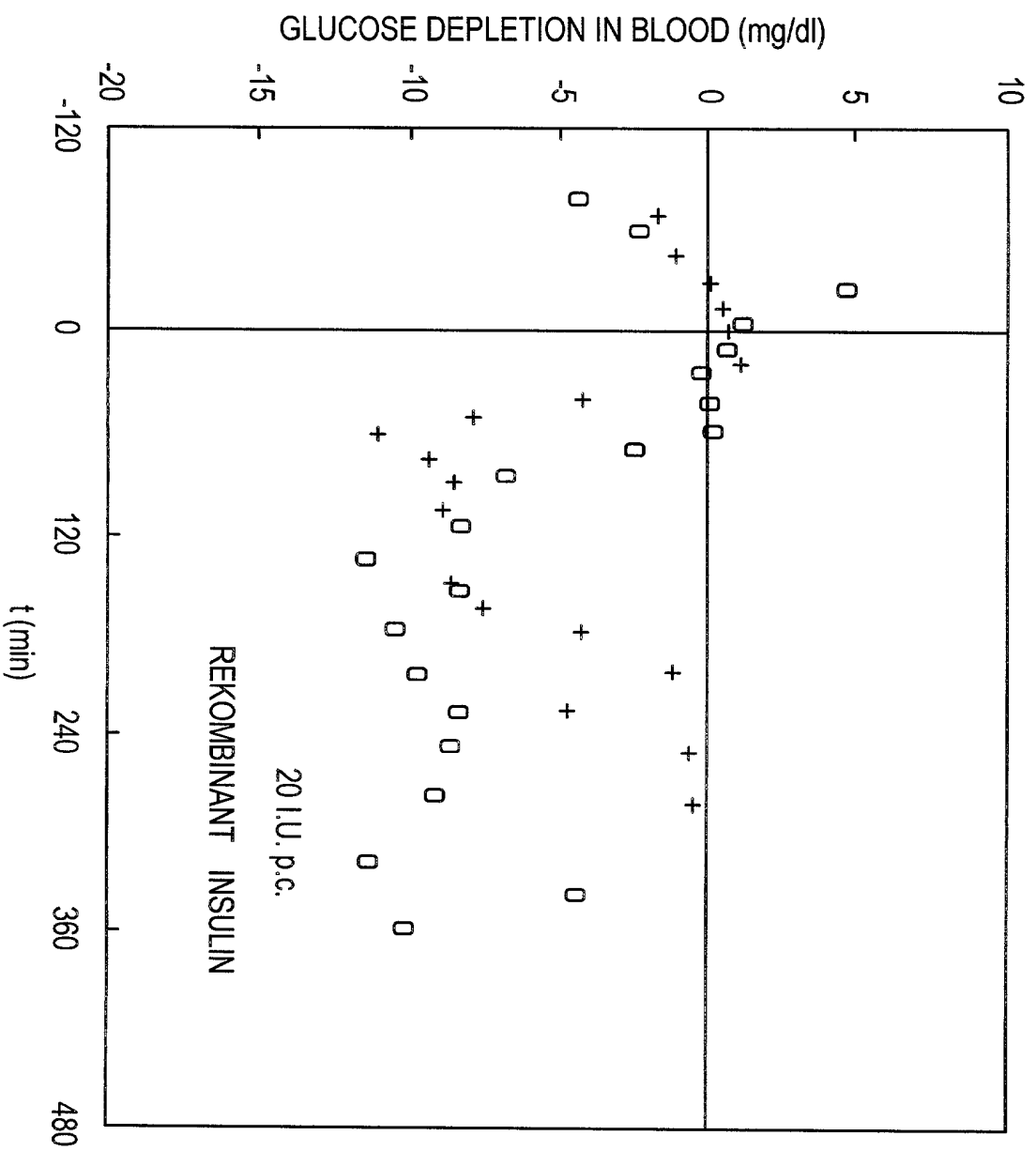


FIG. 14

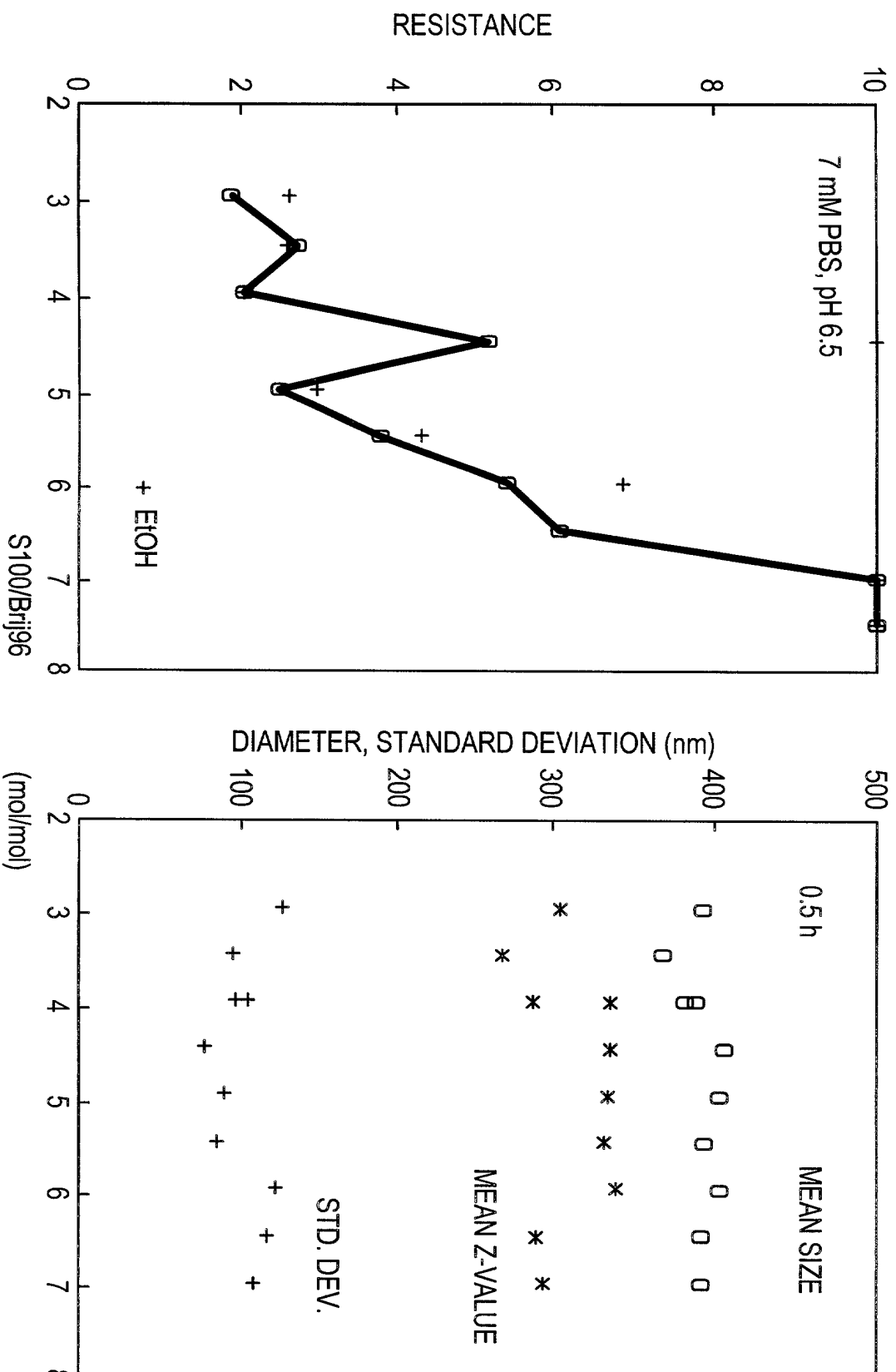


FIG. 15

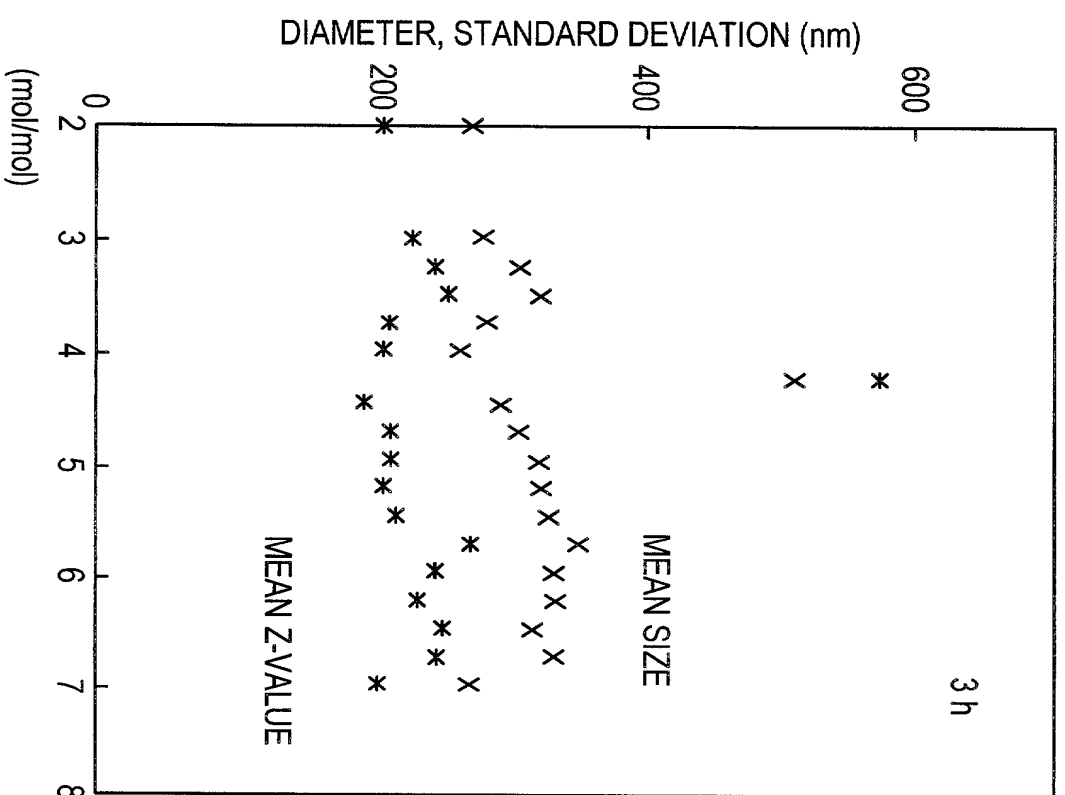
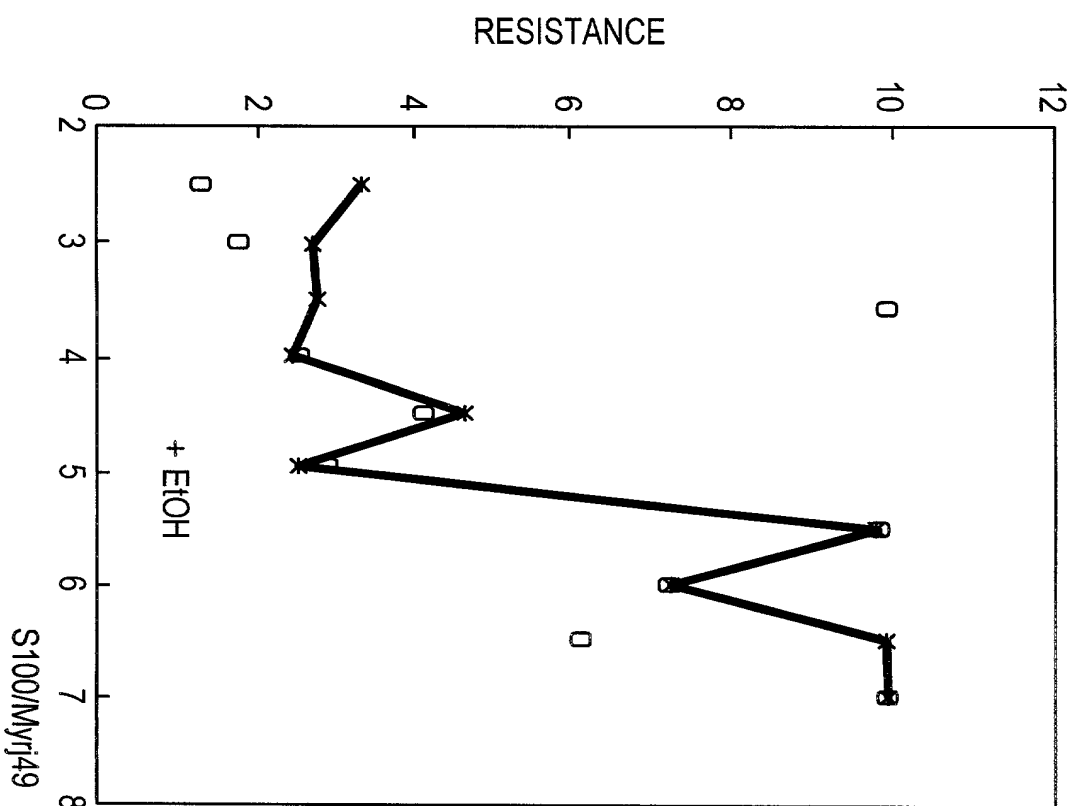


FIG. 16

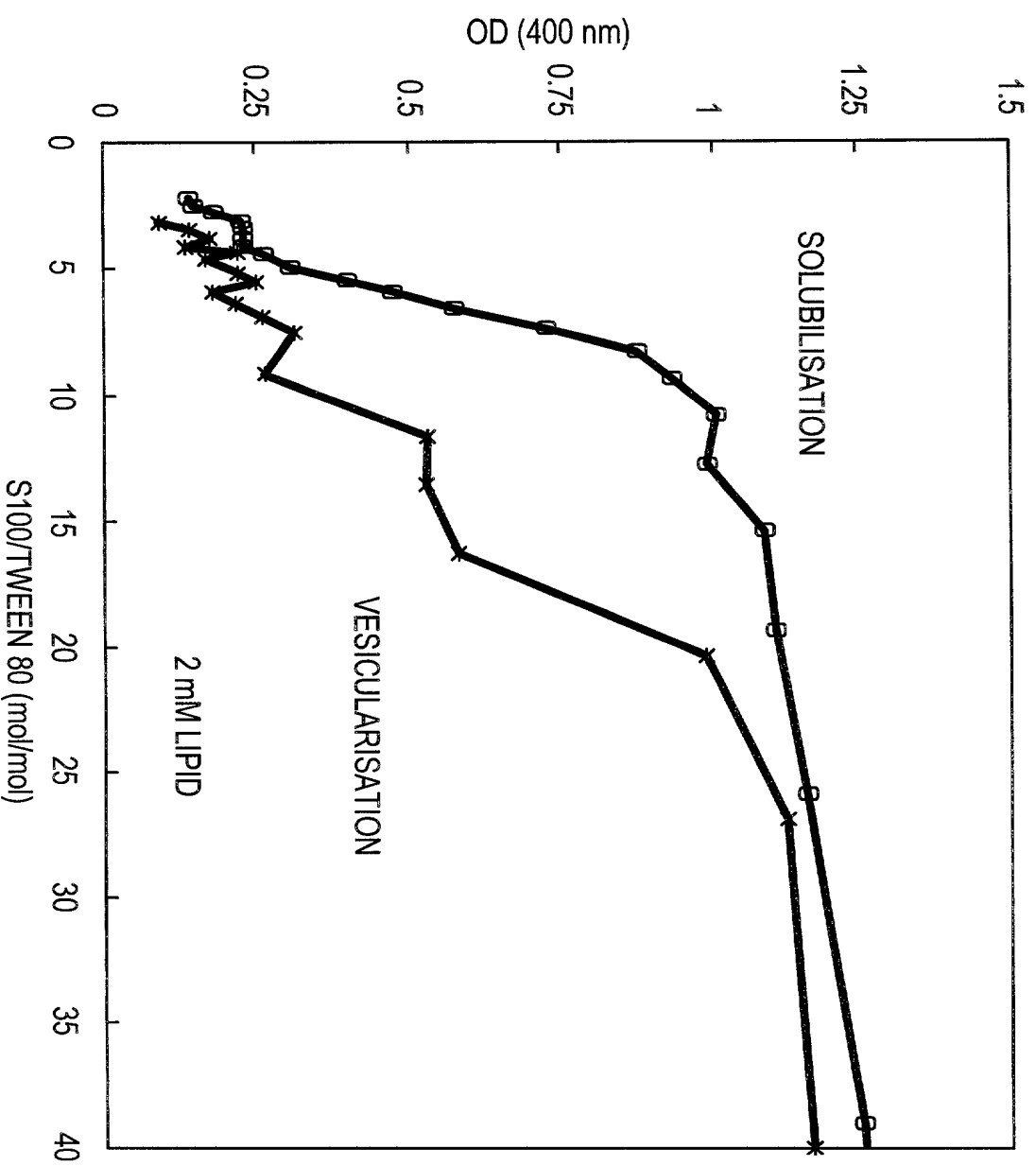


FIG. 17

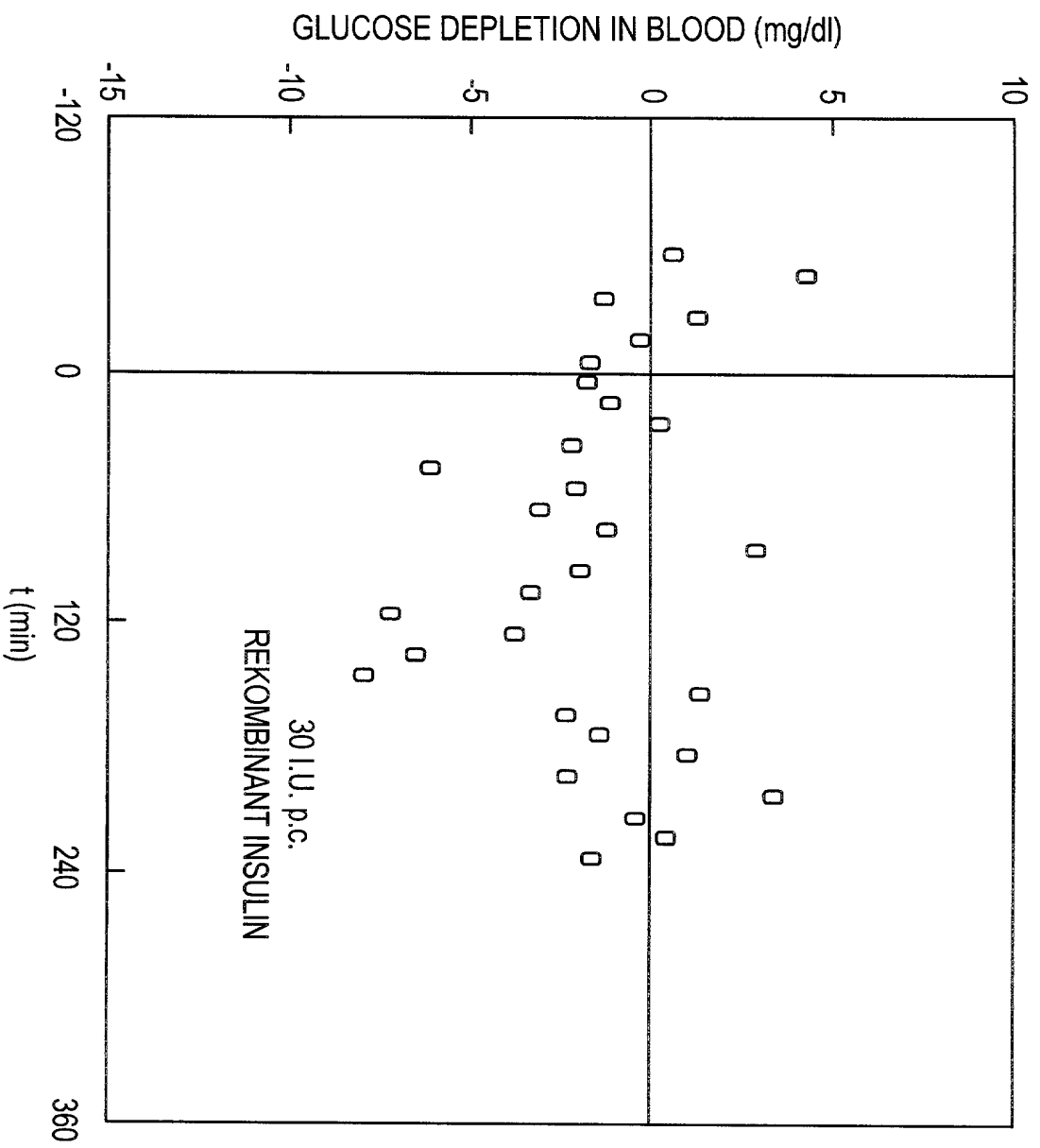


FIG. 18

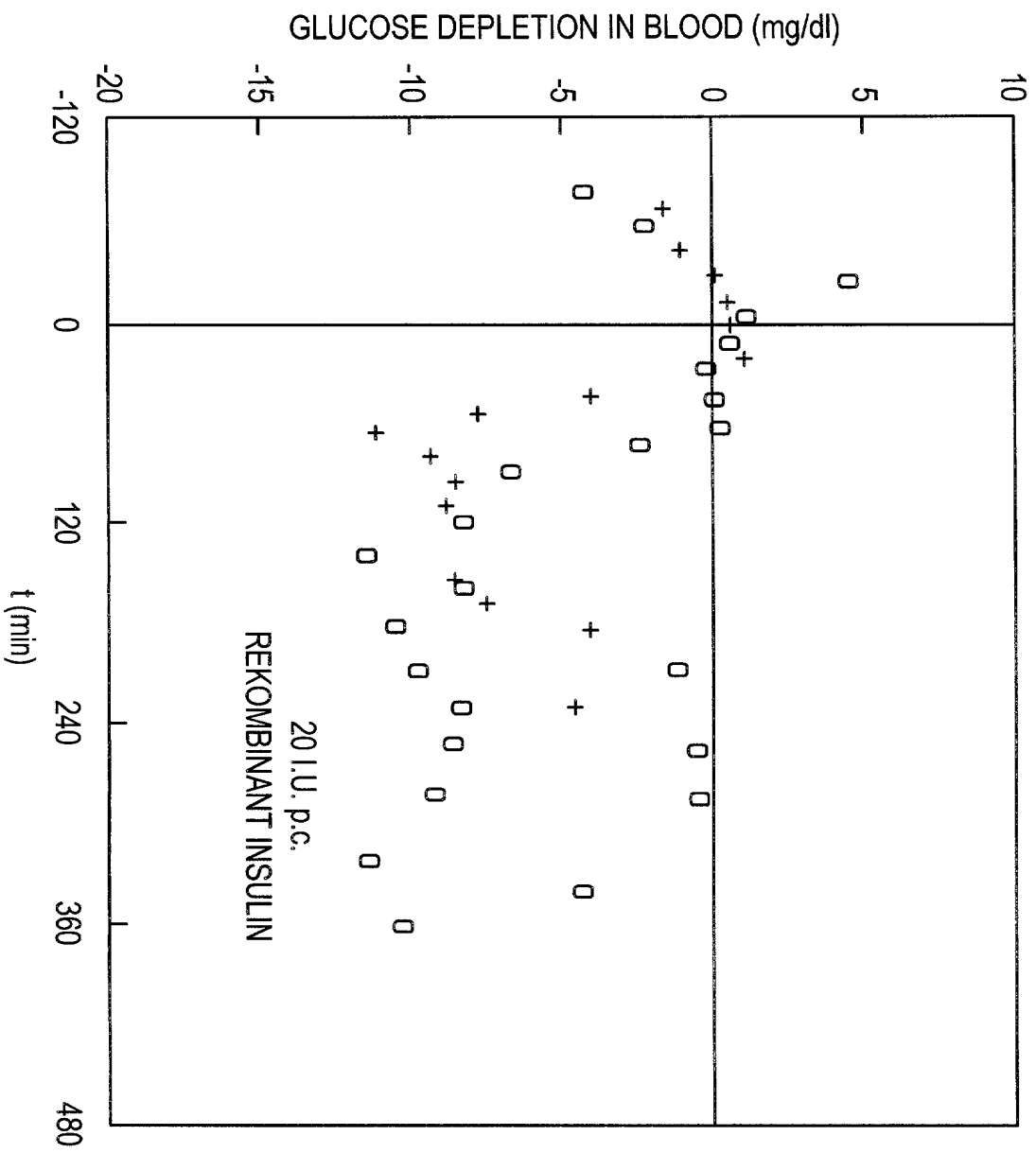


FIG. 19

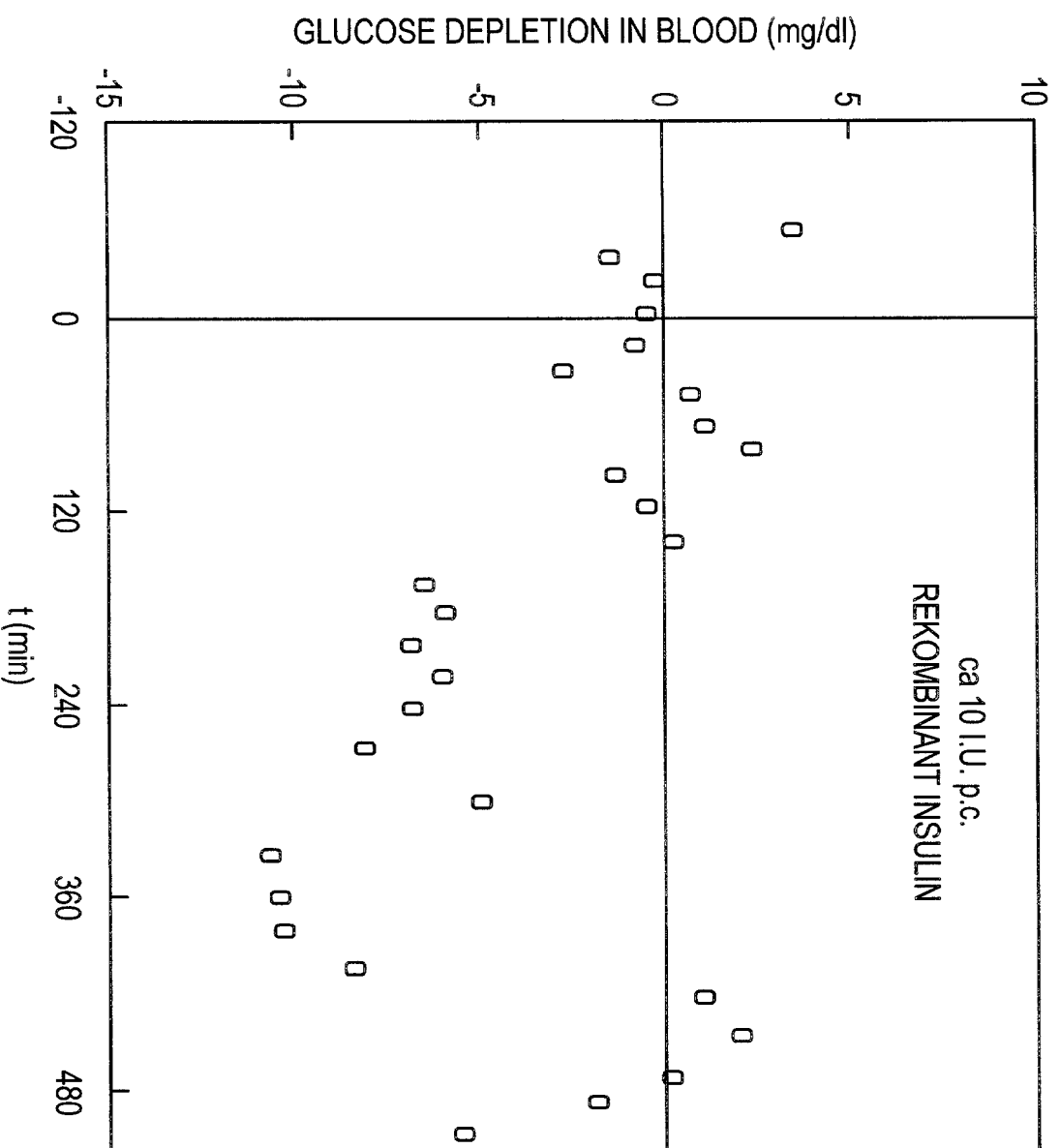


FIG. 20

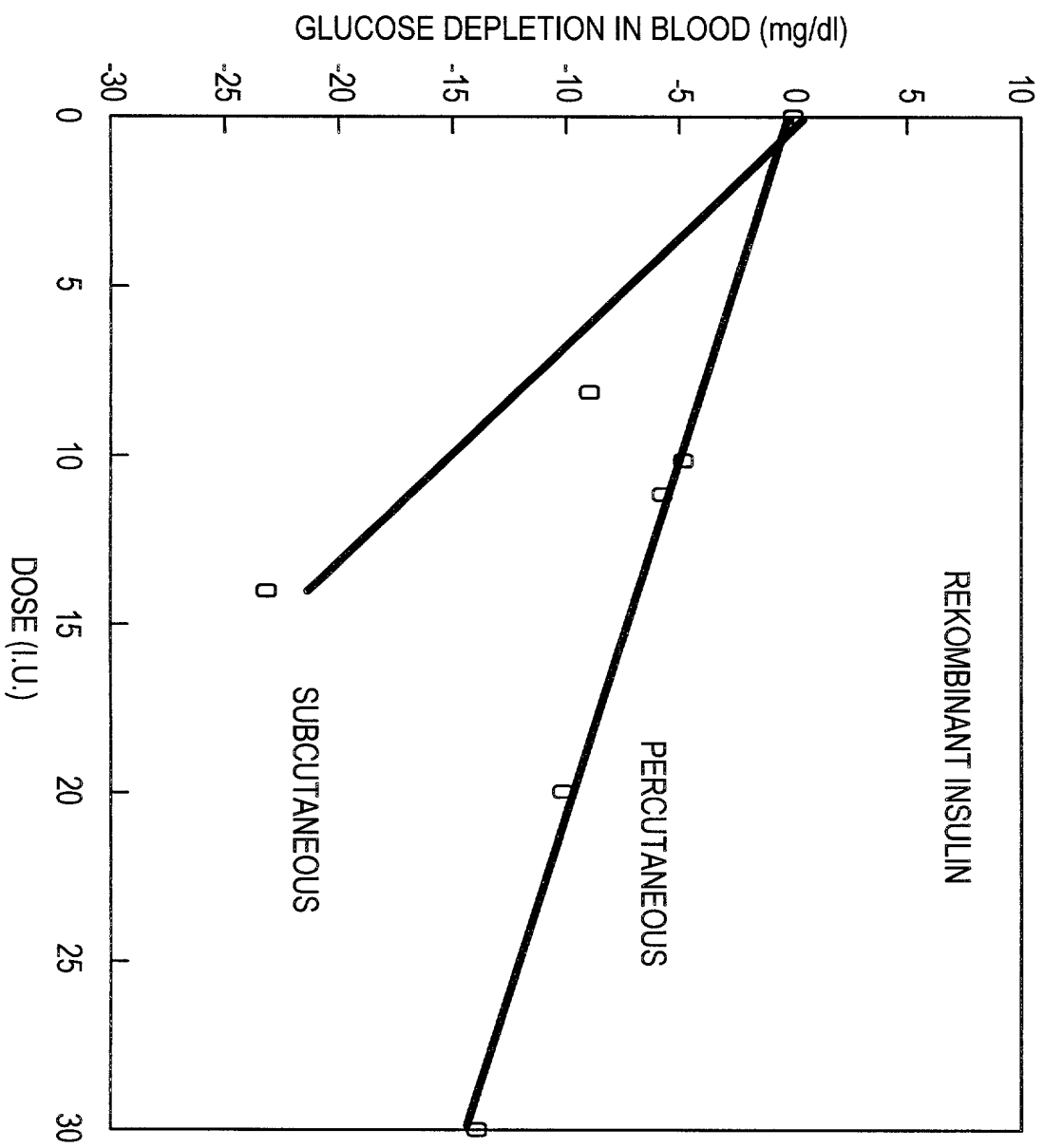
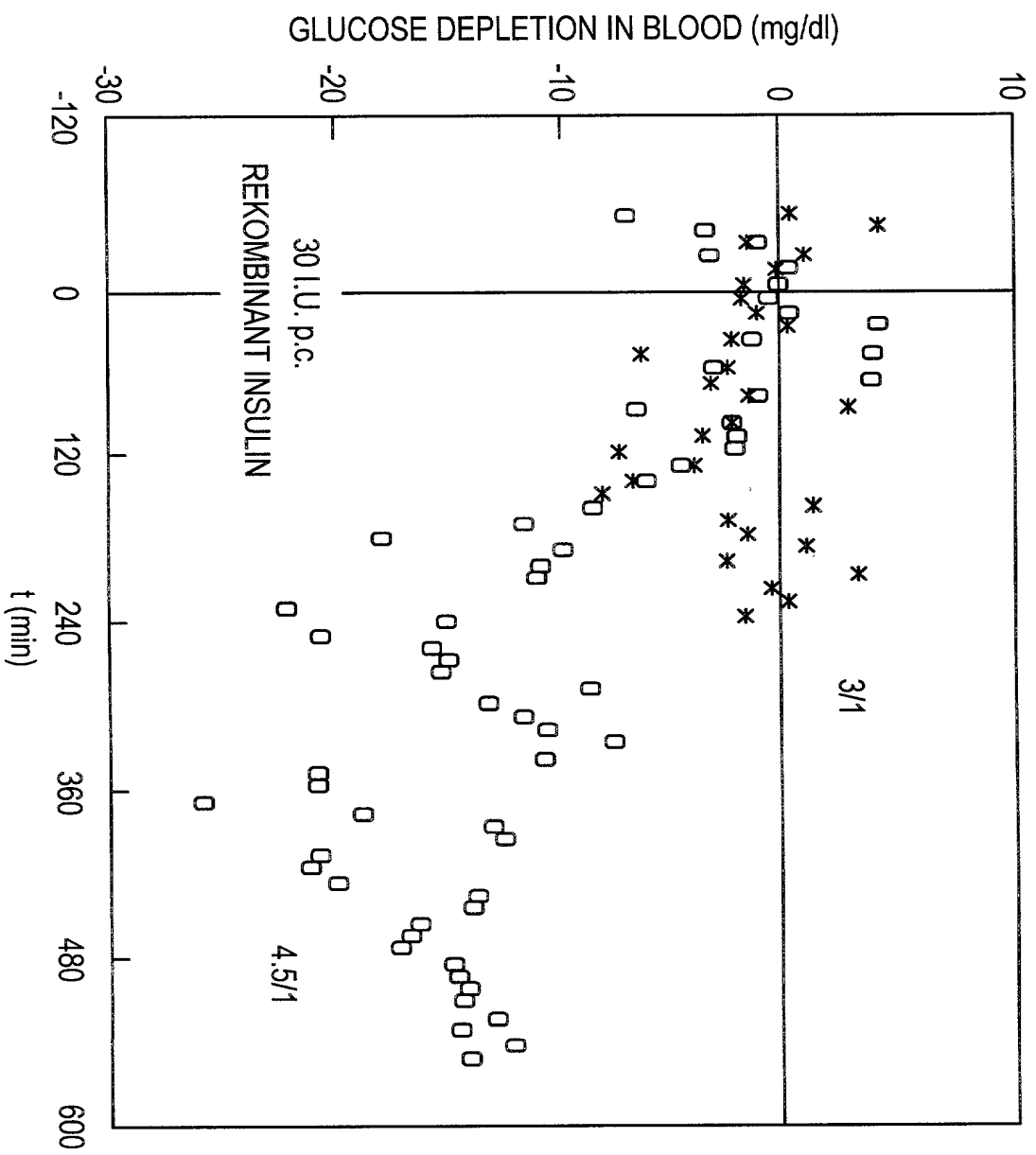


FIG. 21



DECLARATION AND POWER OF ATTORNEY

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PREPARATION FOR THE APPLICATION OF AGENTS IN MINI DROPLETS

_____ is attached hereto

X was filed on April 8, 1992 as Application 07/844,664 and was amended on December 23, 1998; February 26, 1999; October 25, 1999; January 14, 2000; and January 26, 2000 (if applicable).

I hereby authorize and request our attorney, Davidson, Davidson & Kappel, LLC, of 1140 Avenue of the Americas, New York, New York 10036 to insert here in parentheses (Application number _____, filed _____) the filing date and application number of said application when known.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information which is known to me to be material to the patentability of this application as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign and/or provisional application(s) for patent or inventor's certificate listed below and have also identified below any foreign and/or provisional application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR APPLICATION(S)

<u>4026833</u>	<u>Germany</u>	<u>24 August 1990</u>	<u>X</u>	
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
<u>4026834</u>	<u>Germany</u>	<u>24 August 1990</u>	<u>X</u>	
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
<u>4107153</u>	<u>Germany</u>	<u>6 March 1991</u>	<u>X</u>	
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
<u>4107152</u>	<u>Germany</u>	<u>6 March 1991</u>	<u>X</u>	
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

<u>PCT/EP91/01596</u>	<u>22 August 1991</u>	<u></u>
(Application Serial Number)	(Filing Date)	(Status) (patented, pending, abandoned)
<u></u>	<u></u>	<u></u>
(Application Serial Number)	(Filing Date)	(Status) (patented, pending, abandoned)

And I hereby appoint Clifford M. Davidson, Registration No. 32,728, Leslye B. Davidson, Registration No. 38,854, Cary S. Kappel, Registration No. 36,561, William C. Gehris, Registration No. 38,156, Julie L. Bowker, Registration No. 37,870, Money B. Wildes, Registration No. 36,968, Robert J. Paradiso, Registration No. 41,240, Scott L. Appelbaum, Registration No. 41,587, Cynthia R. Moore, Registration No. 46,086 and David Knasiak, Registration No. 45,991 my attorneys, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith; correspondence address: DAVIDSON, DAVIDSON & KAPPEL, LLC, 1140 Avenue of the Americas, 15th Floor, New York, New York 10036; Telephone: (212) 997-1028; Fax: (212) 997-1037.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature Gregor Cevc
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Post Office Address: Same as above

Full name of joint
Inventor, if any _____

Second Inventor's signature _____

Date _____

Residence (city) _____ (state or country) _____

Citizenship _____

Post Office Address: _____